

Supplementary Information

Targeting intrinsically disordered regions facilitates discovery of T-type/Cav3.2 inhibitory peptides for AAV-mediated analgesia

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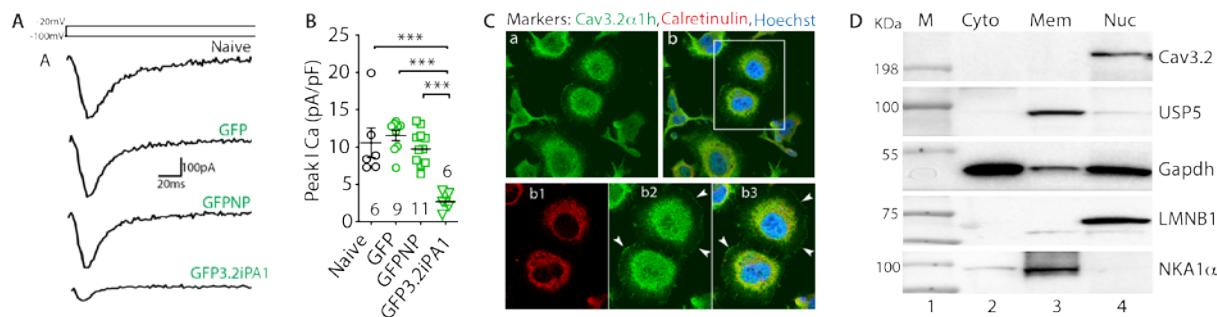


Figure S1. GFP3.2iPA1 inhibits Cav3.2/T-type current in NG-108 cells. Representative T-current traces (A, $V_h = -100$ mV and test -20 mV) and peak I_{Ca} (B) in naïve, GFP-, GFP3.2NP- and GFP3.2iPA1-transfected NG-108 cells (number in each bar is the cells recorded). Mean \pm SEM, *** $p<0.001$, one-way ANOVA and Turkey *post hoc*. Cav3.2 is expressed in NG108 cells with multiple cellular localization (C). Inset in panel b is magnified and shown as montage images (b1-b3) displaying Cav3.2 IR signals localized in cell membrane (b2 and b3, arrowheads) and endoplasmic reticulum (ER, calretininulin-labeled). Cav3.2 is detected in nucleus upon western blots (D).

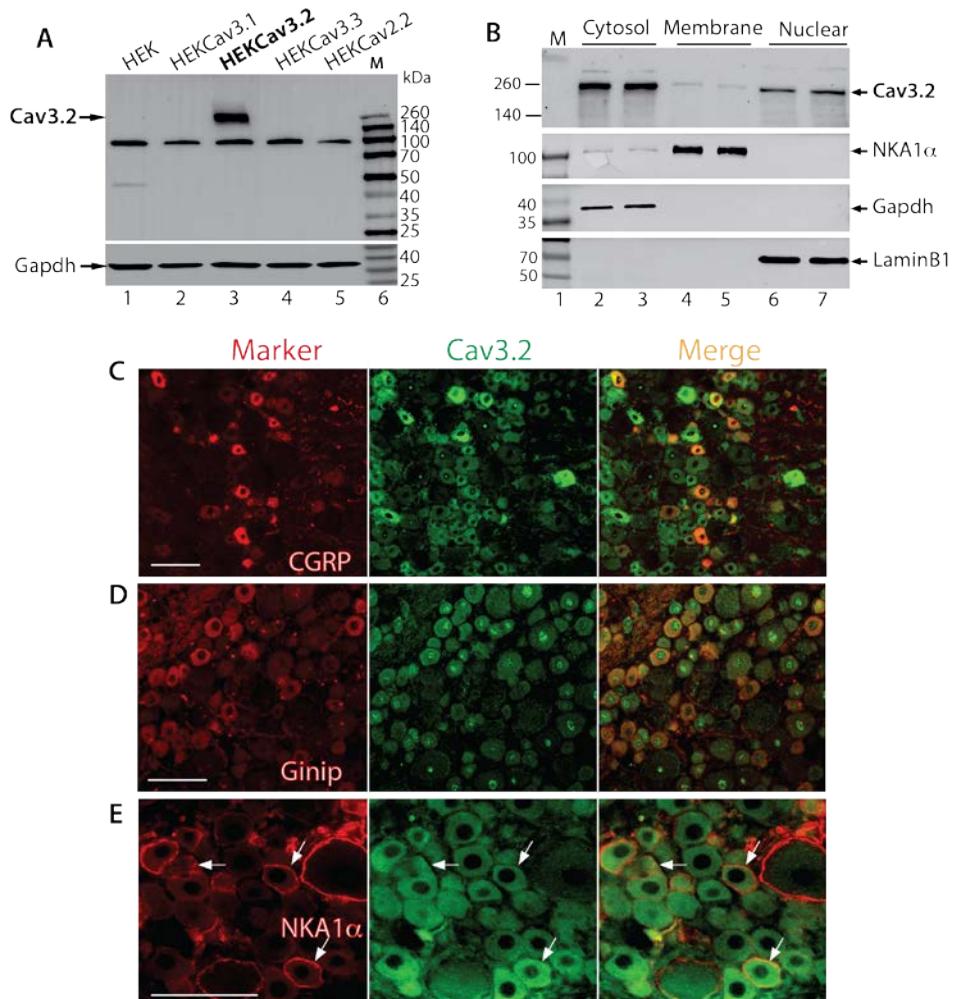


Figure S2. Specificity of Cav3.2 antibody. Immunoblots upon the lysates of cultural HEK cell lines verify that Cav3.2 antibody specifically detects Cav3.2 but not recognizes other T-type calcium channel isoforms and Cav2.2 (**A**), and that Cav3.2 shows multiple cellular localization (cytosol, membrane, and nuclear) prepared from DRG of naïve rats (**B**). Montage IHC images on DRG sections identify small- and medium-sized sensory neurons expression profile of Cav3.2, colabeling with CGRP and Ginip (**C**, **D**). Cav3.2 and NKA1 α colabeling shows Cav3.2 identification in the PM of sensory neuron (**E**). Scale bars: 50 μ m for all IHC images.

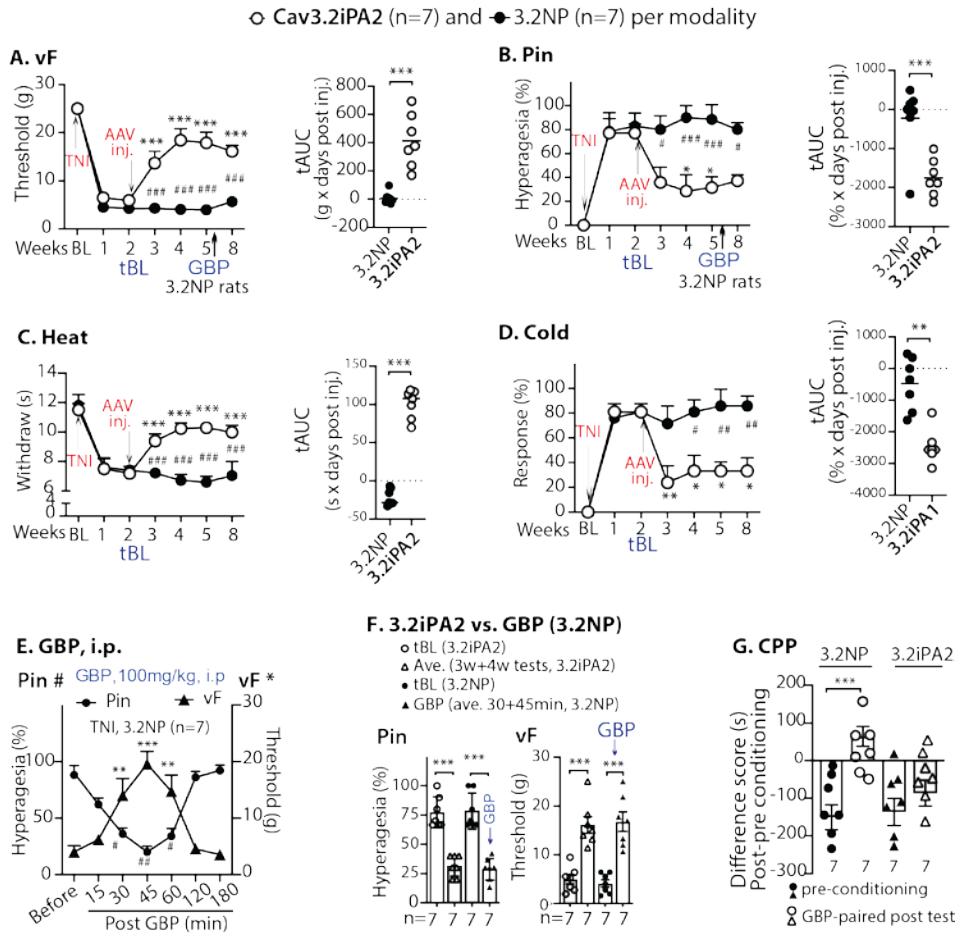
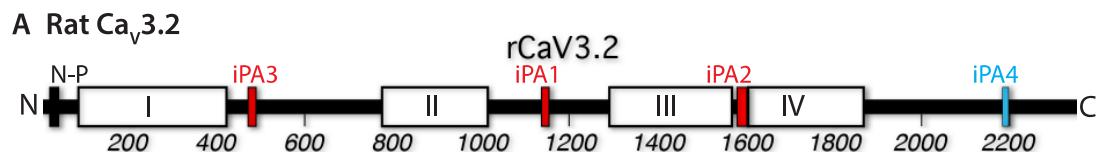


Figure S3. Treatment of established neuropathic pain by DRG delivery of AAV6-3.2iPA2 (male rats). Analogous figures to Fig. 6 for treatment with AAV6-3.2iPA2 shows comparable effectiveness to established TNI pain likewise to AAV6-3.2iPA1.



B SeqNLS (nuclear localization sequence) prediction

	aa	aa sequence	aa	NLS Score	Definition	Score	Color
NP	22	PAAPVRASPASPGAPGREQ	41	0.0	0.1 - 0.3		
iPA3	472	KRRSLRLYARWQSRWRKK	489	0.960	0.3 - 0.5		
iPA1	1136	SSRRSSWNNSLGRAPSLKRR	1154	0.960	0.5 - 0.7		
iPA2	1582	RRREEKRLRRRLERRRKQAQRRP	1603	0.960	0.7 - 0.8		
iPA4	2176	RGRASELEPALGSRRKKKMSPP	2197	0.630	0.8 - 0.86		
					0.86 - 0.89		
					>0.89		

Figure S4. *In silico* prediction of nuclear localization signals (NLS) of Cav3.2iPAs. (A) Diagram of full length of Cav3.2 protein, with white boxes labeled DI-DIV of Cav3.2 TM domain I-IV and the bars within the sequences showing the position of the predicted iPAs. (B) NLS predicted by use of SeqNLS (<http://mleg.cse.sc.edu/seqNLS/>), with the cutoff score > 0.86 for a potential NLS.

Suppl. Table 1 (exact p values for all figures)

Figure 2.

Figure 2I (Ica peak) (MCW)	Exact p value	Figure 2J (Act. V2/1)	Exact p value
Sham vs. GFP	0.7479	Sham vs. GFP	0.3941
Sham vs. 3.2NP	>0.9999	Sham vs. 3.2NP	0.7890
Sham vs. 3.2iPA3	>0.9999	Sham vs. 3.2iPA1	0.1972
Sham vs. 3.2iPA1	<0.0001	Sham vs. 3.2iPA2	0.9630
Sham vs. 3.2iPA2	<0.0001	Figure 2I (inact. V2/1)	
Sham vs. 3.2iPA4	0.0002	Sham vs. GFP	>0.9999
GFP vs. 3.2NP	0.9063	Sham vs. 3.2NP	0.1753
GFP vs. 3.2iPA3	0.9391	Sham vs. 3.2iPA1	0.6397
GFP vs. 3.2iPA1	<0.0001	Sham vs. 3.2iPA2	0.1627
GFP vs. 3.2iPA2	<0.0001		
GFP vs. 3.2iPA4	0.021		
3.2NP vs. 3.2iPA1	<0.0001		
3.2NP vs. 3.2iPA2	<0.0001		
3.2NP vs. 3.2iPA4	0.0004		

Figure 2.

Figure 2H1 (Ica peak) (SBU)	Exact p	Figure 2H1 (Act. V2/1)	Exact p value
Sham vs. GFP	>0.9999	Sham vs. GFP	>0.9999
Sham vs. 3.2NP	0.9359	Sham vs. 3.2NP	>0.9999
Sham vs. 3.2iPA1	<0.0001	Sham vs. 3.2iPA1	0.417
Sham vs. 3.2iPA2	<0.0001	Sham vs. 3.2iPA2	0.9858
GFP vs. 3.2NP	0.956	Figure 2I1 (inact. V2/1)	
GFP vs. 3.2iPA1	0.0003	Sham vs. GFP	0.1133
GFP vs. 3.2iPA2	<0.0001	Sham vs. 3.2NP	>0.9999
3.2NP vs. 3.2iPA1	0.0034	Sham vs. 3.2iPA1	>0.9999
3.2NP vs. 3.2iPA2	<0.0001	Sham vs. 3.2iPA2	>0.9999

Figure 3.

Figure 3B5 (Ica3.1 peak)	Exact p value	Figure 3D5 (Ica3.3 peak)	Exact p value
Sham vs. 3.2NP	0.9980	Sham vs. 3.2NP	0.9974
Sham vs. 3.2iPA1	0.0055	Sham vs. 3.2iPA1	0.9919
Sham vs. 3.2iPA2	0.0220	Sham vs. 3.2iPA2	>0.9999
3.2NP vs. 3.2iPA1	0.0095	3.2NP vs. 3.2iPA1	0.9997
3.2NP vs. 3.2iPA2	0.0350	3.2NP vs. 3.2iPA2	0.9945
Figure 3C5 (Ica3.2 peak)	Exact p value		
Sham vs. 3.2NP	0.9085		
Sham vs. 3.2iPA1	<0.0001		
Sham vs. 3.2iPA2	<0.0001		
3.2NP vs. 3.2iPA1	<0.0001		
3.2NP vs. 3.2iPA2	<0.0001		

Figure 4.

Figure 4A8 (I_{Na1.7} peak)	Exact p value	Figure 4C8 (Ca2+ imaging)	Exact p value
Sham vs. GFP	0.9525	Sham vs. 3.2iPA1	<0.0001
Sham vs. 3.2NP	0.4632	Sham vs. TTAp2	<0.0001
Sham vs. 3.2iPA1	0.7081	Figure 4D8 (HVA Ica peak)	Exact p value
Sham vs. 3.2iPA2	0.7780	Sham vs. GFP	>0.9999
Figure 4B8 (I_k peak)	Exact p value	Sham vs. 3.2NP	0.9868
Sham vs. GFP	0.9650	Sham vs. 3.2iPA1	0.9993
Sham vs. 3.2NP	0.9665	Sham vs. 3.2iPA2	0.9993
Sham vs. 3.2iPA1	0.9855		
Sham vs. 3.2iPA2	0.8799		
Figure 4C7 (LVA Ica peak)	Exact p value		
Sham vs. 3.2NP	0.9054		
Sham vs. 3.2iPA3	0.0230		
Sham vs. 3.2iPA1	<0.0001		
Sham vs. 3.2iPA2	<0.0001		
3.2NP vs. 3.2iPA3	0.0025		
3.2NP vs. 3.2iPA1	<0.0001		
3.2NP vs. 3.2iPA2	<0.0001		

Figure 5.

Figure 5F

Figure 5C (Ica peak)	Exact p value	Figure 5D	Exact p value
Sham vs. TNI	<0.0001	Sham vs. TNI (Cav3.2)	0.0047
		Sham vs. TNI (GFAP)	0.0276

Figure 5G (vF)

NP, BL vs. 1w	NP, BL vs. 2w	NP, BL vs. 3w	NP, BL vs. 4w	NP, BL vs. 5w
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
3.2iPA1, BL vs.	3.2iPA1, BL vs.	3.2iPA1, BL vs. 3w	3.2iPA1, BL vs. 4w	3.2iPA1, BL vs. 5w
<0.0001	<0.0001	0.0025	0.4546	0.0405
1w NP vs. 3.2iPA1	2w NP vs. 3.2iPA1	3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1
>0.9999	0.0025	<0.0001	<0.0001	<0.0001
vF AUC (NP vs. 3.2iPA1)				
0.0006				

Figure 5H (Pin)

NP, BL vs. 1w	NP, BL vs. 2w	NP, BL vs. 3w	NP, BL vs. 4w	NP, BL vs. 5w
0.0018	0.0017	<0.0001	<0.0001	<0.0001
3.2iPA1, BL vs. 1w	3.2iPA1, BL vs. 2w	3.2iPA1, BL vs. 3w	3.2iPA1, BL vs. 4w	3.2iPA1, BL vs. 5w
0.0014	0.0133	0.0440	0.0370	0.1528
1w NP vs. 3.2iPA1	2w NP vs. 3.2iPA1	3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1
>0.9999	0.3135	0.4479	0.1188	0.048
Pin AUC (NP vs. 3.2iPA1)				
0.0026				

Figure 5I (Brush)

NP, BL vs. 1w	NP, BL vs. 2w	NP, BL vs. 3w	NP, BL vs. 4w	NP, BL vs. 5w
0.0129	0.0435	0.0180	0.0033	0.0001
3.2iPA1, BL vs.				

0.0101	0.012	0.1651	0.3254	>0.9999
1w NP vs. 3.2iPA1	2w NP vs. 3.2iPA1	3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1
>0.9999	>0.9999	>0.9999	0.5967	0.0125
Pin AUC (NP vs. 3.2iPA1)				
0.062				

Figure 5J (Heat)

NP, BL vs. 1w	NP, BL vs. 2w	NP, BL vs. 3w	NP, BL vs. 4w	NP, BL vs. 5w
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
3.2iPA1, BL vs. 1w	3.2iPA1, BL vs. 2w	3.2iPA1, BL vs. 3w	3.2iPA1, BL vs. 4w	3.2iPA1, BL vs. 5w
<0.0001	<0.0001	0.0404	0.9570	>0.9999
1w NP vs. 3.2iPA1	2w NP vs. 3.2iPA1	3w NP vs. 3.2iPA1	4w NP vs.	5w NP vs. 3.2iPA1
>0.9999	0.5382	0.0020	<0.0001	<0.0001
AUC NP vs iPA1				
0.0008				

Figure 5K (Cold)

NP, BL vs. 1w	NP, BL vs. 2w	NP, BL vs. 3w	NP, BL vs. 4w	NP, BL vs. 5w
0.0030	0.0027	0.005	0.0027	0.0032
3.2iPA1, BL vs. 1w	3.2iPA1, BL vs. 2w	3.2iPA1, BL vs. 3w	3.2iPA1, BL vs. 4w	3.2iPA1, BL vs. 5w
0.0010	0.0871	0.4764	0.2851	>0.9999
1w NP vs. 3.2iPA1	2w NP vs. 3.2iPA1	3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1
>0.9999	>0.9999	0.1593	0.7191	0.1262
AUC NP vs iPA1				
0.004				

Figure 6.

Figure 6C (vF)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA1, tBL vs. 3w	3.2iPA1, tBL vs. 4w	3.2iPA1, tBL vs. 5w	3.2iPA1, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1	8w NP vs. 3.2iPA1
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA1			
<0.0001			

Figure 6D (Pin)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA1, tBL vs. 3w	3.2iPA1, tBL vs. 4w	3.2iPA1, tBL vs. 5w	3.2iPA1, tBL vs. 8w
0.0035	0.015	0.019	0.025
3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1	8w NP vs. 3.2iPA1
0.0138	0.0014	0.0126	0.0190
AUC NP vs iPA1			
0.0006			

Figure 6E (Heat)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA1, tBL vs. 3w	3.2iPA1, tBL vs. 4w	3.2iPA1, tBL vs. 5w	3.2iPA1, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1	8w NP vs. 3.2iPA1
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA1			
0.0002			

Figure 6F (Cold)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA1, tBL vs. 3w	3.2iPA1, tBL vs. 4w	3.2iPA1, tBL vs. 5w	3.2iPA1, tBL vs. 8w
0.0072	0.0017	0.0062	0.0183
3w NP vs. 3.2iPA1	4w NP vs. 3.2iPA1	5w NP vs. 3.2iPA1	8w NP vs. 3.2iPA1
0.0453	0.0019	0.0069	0.0206
AUC NP vs iPA1			
0.0006			

Figure 6G (GBP, i.p.)

CBP: before vs. 30min post (vF)	CBP: before vs. 45min post (vF)	CBP: before vs. 60min post (vF)
0.0060	<0.0001	0.0076
CBP: before vs. 30min post (Pin)	CBP: before vs. 45min post (Pin)	CBP: before vs. 60min post (Pin)
0.0010	0.0021	0.0640

Figure 6H (GBP vs. 3.2iPA1)

3.2iPA1, tBL vs. 4w+5w post inj. (pin)	NP, tBL vs. 30~60 min post GBP inj. (pin)	3.2iPA1, tBL vs. 4w+5w post inj. (vF)	NP, tBL vs. 30~60 min post GBP inj. (vF)
0.0001	0.0006	<0.0001	0.0006

Figure 6I (CPP)

NP, pre-conditioning vs. GBP paired	3.2iPA1, pre-conditioning vs. GBP paired post test
0.0007	0.9015

Figure 8.

Figure 8C (Main effects)

Sham vs. TNI	Sham vs. TNI+NP	Sham vs. TNI+3.2iPA1	TNI vs. TNI+NP	TNI vs. TNI+3.2iPA1	TNI+NP vs. TNI+3.2iPA1
<0.0001	0.0011	0.7823	0.5545	<0.0001	<0.0001

Figure 8D (Rheobase)

Sham vs. TNI	Sham vs. TNI+3.2NP	Sham vs. TNI+3.2iPA1	TNI vs. TNI+NP	TNI vs. TNI+3.2iPA1	NP+TNI vs. TNI+3.2iPA1
<0.0001	<0.0001	0.8856	0.9349	0.0005	0.0112

Figure 8E (0.5nA)

Sham vs. TNI	Sham vs. TNI+3.2NP	Sham vs. TNI+3.2iPA1	TNI vs. TNI+NP	TNI vs. TNI+3.2iPA1	NP+TNI vs. TNI+3.2iPA1
<0.0001	<0.0001	0.9996	0.9349	<0.0001	<0.0001

Figure 8E1 (1.0nA)

Sham vs. TNI	Sham vs. TNI+3.2NP	Sham vs. TNI+3.2iPA1	TNI vs. TNI+NP	TNI vs. TNI+3.2iPA1	NP+TNI vs. TNI+3.2iPA1
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<0.0001	<0.0001	0.6569	0.9059	<0.0001	<0.0001
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Figure 9.

Figure 9A (vF)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	0.9986	0.9989
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA2			
<0.0001			

Figure 9B (Pin)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
0.0008	0.0008	0.0025	0.0032
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
0.0055	0.0020	0.0227	0.0139
AUC NP vs iPA2			
<0.0001			

Figure 9C (Heat)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
0.3130	0.9443	0.9871	0.9755
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w NP vs. 3.2iPA2	4w NP vs. 3.2iPA2	5w NP vs. 3.2iPA2	8w NP vs. 3.2iPA2
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA2			
<0.0001			

Figure 9D (Cold)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	0.9986	0.9989
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w NP vs. 3.2iPA2	4w NP vs. 3.2iPA2	5w NP vs. 3.2iPA2	8w NP vs. 3.2iPA2
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA2			
0.0001			

Figure 9E (CPP)

NP, pre-conditioning vs. GBP paired	3.2iPA2, pre-conditioning vs. GBP paired post test
0.0091	0.2732

Suppl. Figure 3

Suppl. Figure 3A (vF)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	0.9416

3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA2			
0.0001			

Suppl. Figure 3B (Pin)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
0.0521	0.0151	0.0194	0.0650
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
0.0305	0.0001	0.0004	0.0287
AUC NP vs iPA2			
0.0001			

Suppl. Figure 3C (Heat)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
0.9908	0.2825	0.1318	0.8939
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
<0.0001	<0.0001	<0.0001	<0.0001
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
<0.0001	<0.0001	<0.0001	<0.0001
AUC NP vs iPA2			
<0.0001			

Suppl. Figure 3D (Cold)

NP, tBL vs. 3w	NP, tBL vs. 4w	NP, tBL vs. 5w	NP, tBL vs. 8w
>0.9999	>0.9999	>0.9999	>0.9999
3.2iPA2, tBL vs. 3w	3.2iPA2, tBL vs. 4w	3.2iPA2, tBL vs. 5w	3.2iPA2, tBL vs. 8w
0.0068	0.0180	0.0133	0.0133
3w, NP vs. 3.2iPA2	4w, NP vs. 3.2iPA2	5w, NP vs. 3.2iPA2	8w, NP vs. 3.2iPA2
0.0510	0.0245	0.0073	0.0073
AUC NP vs iPA2			
0.0012			

Suppl. Figure 3E (GBP, i.p)

CBP: before vs. 30min post (vF)	CBP: before vs. 45min post (vF)	CBP: before vs. 60min post (vF)
0.0093	<0.0001	0.0067
CBP: before vs. 30min post (Pin)	CBP: before vs. 45min post (Pin)	CBP: before vs. 60min post (Pin)
0.0308	0.0019	0.0253

Suppl. Figure 3F (GBP vs. 3.2iPA2)

3.2iPA1, tBL vs. 4w+5w post inj. (pin)	NP, tBL vs. 30~60 min post GBP inj. (pin)	3.2iPA1, tBL vs. 4w+5w	NP, tBL vs. 30~60 min post GBP inj. (vF)
<0.0001	<0.0001	<0.0001	<0.0001

Suppl. Figure 3G (CPP)

NP, pre-conditioning vs. GBP paired	3.2iPA1, pre-conditioning vs. GBP paired post test
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0.0003	0.1961
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