

Supplemental Information

Supplementary Figure 1. Demonstration of the effectiveness of EMT elicited via the treatment of parental HK2 cells with TGF β 1 for 3 days in culture. Parental HK2 cells show a cobblestone morphology characteristic of epithelial cells, robust ZO-1 staining at the cell-cell junctions and no appreciable FSP-1 protein. Note also the lack of PAX2 protein expression suggesting that these cells are not under stress. After TGF β 1 treatment, the cells adopted an elongated fibroblastic phenotype, lost expression of ZO-1, upregulated FSP-1 and showed a relocation of F-actin filaments away from the sub-cortical regions of the cells.

Supplementary Figure 2. Evidence that Pool 8 reprogramming factors can upregulate NP gene and protein expression in human primary renal proximal tubule epithelial cells (RPTECs). **A.** qRT-PCR analysis of RPTEC cells cultured with (blue and red bars) or without (black bars) VPA and transduced with either GFP-only lentivirus (black and blue bars) or Pool 8 factors (red bars). Data represents the average of 3 replicates with each gene normalized to *GAPDH* and expressed relative to RPTEC–VPA. Error bars represent SEM. **B.** Immunofluorescence of control (RPTEC–VPA) cells compared to RPTECs exposed to Pool 8 reprogramming factors, showing expression of GFP (green) and SIX2 (red) protein. **C.** qRT-PCR analysis of *PAX2* expression in primary human RPTECs compared to immortalised HK2 cells. Data represents the average of 3 replicates, with expression normalized to *GAPDH* and RPTEC expressed relative to HK2. Error bars represent SEM.

Supplementary Figure 3. Immunofluorescence for PAX2 protein (red) in HK2 parental, HK2+VPA and HK2+Pool 8. Co-immunofluorescence for GFP (green) illustrates the association between PAX2 protein and lentiviral infection. DAPI (blue). Scale bar = 30 μ m.

Supplementary Figure 4. Immunofluorescence for CITED1 protein (red) in HK2 parental, HK2+VPA and HK2+Pool 8. Co-immunofluorescence for GFP (green) illustrates the association between CITED1 protein and lentiviral infection. DAPI (blue). Scale bar = 30 μ m.

Supplementary Figure 5. Evidence that the recombination assay recapitulates embryonic kidney development. Sections from E15.5 kidney or from recombinations between wildtype unlabelled E12.5 mouse embryonic kidneys are shown stained with antibodies to ureteric epithelium (Calbindin 28kD, Calb), Pax2 (marks ureteric epithelium nephron progenitor and forming nephrons), Wt1 (marks nephron progenitor and forming nephrons), Aqp1 (marks proximal tubules) and laminin (marks basement membranes around epithelial structures). Cells within recombinations are viable, as evidenced by expression of cell cycle genes Ki67 and pHH3. Scale bar = 500 μ m.

Supplementary Figure 6. A. Lack of incorporation of GFP-labelled cell lines into Wt1+, Six2+ or Calbindin+ compartment within recombination assays. Note that M15 is positive for Wt1 protein in culture. M15: Mouse mesonephric cell line; MSC: murine primary mesenchymal cell line; HEK293T: human embryonic kidney-derived cell line; HK2: human proximal tubule cell line. MSCs were derived from constitutionally GFP+ mice. All other cell lines were marked with GFP via lentiviral infection. **B.** Examples of lack of incorporation of GFP+ HK2 cells either reprogrammed with empty lentivirus in the presence of VPA (VPA) or treated with recombinant TGF β 1 (TGF β 1). Scale bar = 300 μ m.

Supplementary Figure 7. Evidence that HK2 cells transduced with Pool 8 factors proliferate in the recombination assay. Sections from recombinations of E12.5 kidney and HK2+Pool 8

test cells were stained with antibodies against GFP (green) and pHH3 (red). Merged image depicts an example of a transduced HK2 cell showing co-localisation of both GFP and pHH3.

Supplementary Figure 8. Reprogramming with SNAI2+VPA. *SNAI2*-overexpressing cells appear elongated and spindle-shaped and are FSP-1⁺.

Supplementary Table 1. Nested primers for subcloning of candidate reprogramming factors.

Supplementary Table 2. Forward and reverse primers sequences used for qRT-PCR of endogenous gene expression.

Supplementary Table 3. Forward and reverse primer sequences used for RT-PCR.

Supplementary Table 4. Antibodies used for immunofluorescence.

Supplementary Table 1. Nested primers for subcloning of candidate reprogramming factors.

Gene	Source cDNA	Primary (1) and secondary (2) PCR primers for nested PCR (5' – 3')
Six2 (AF136940)	Human fetal kidney	(1) F: GAAGCTCCCATGCGGGAC R: CCAGTGTCAGTCACAAAAGG (2) F: ATGTCCATGCTGCCCACCTTCG R: CTAGGAGCCCAGGTCCACGAGG
Six1 (NM_009189)	Human fetal kidney	(1) F: TCCGTGTCCAGAACCTCCCCCTACTC R: GCTGCTCCAGAAATCCCTTCG (2) F: ATGTGATGCTGCCGTCGTTTG R: TTAGGACCCCAAGTCCACCAGAC
Osr1 (NM_145260)	Human fetal kidney	(1) F: AGAAGCCACTGCAACTACCGR: CCTTGTGACCCACAGGTTCT (2) F: ATGGGCAGCAAAACCTTGR: TTAGCATTTGATCTTGGAGG
Crym (BC018061)	Human fetal kidney	(1) F: TGAGGTTAGAAGGCACAGGTR: CATCCATCTCAACATCAAGTTC (2) F: ATGAGCCGGGTACCAGCGR: TTATTTACCAGATGACCAGGAA
Meox2 (NM_005924)	Mouse embryonic kidney	(1) F: ACCGTCCGTGAAGTCTTCTG R: TCACAATGGTGCTTTCCTGA (2) F: ATGGAACACCCCCTCTTTGGC R: TATCATAAGTGTGCGTGCTCAGAG
Eya1 (NM_172058)	Human fetal kidney	(1) F: GATGTTGCTGTTTCTCAAG R: TCACCAGGCGGAATTGCTAAGTTCTGG (2) F: ATGTTGCTCTTCTCAAGTTGCAR: TTACAGGTACTCCAGTTCCA
Hoxa11 (NM_005523)	Human cDNA clone	(1) na (2) F: ATGGATTTTGATGAGCGTGGTCCR: TTAGAGGAGTGGATTGTC
Wt1 (BC032861)	Human cDNA clone	(1) na (2) F: ATGGAGAAGGGTTACAGCACG R: TCAAAGCGCCAGCTGGAGT
Pax2 (NM_011037)	Mouse embryonic kidney	(1) F: TCTGCCTCCCGATGGATA R: GCCTGAAGCTTGATGTGGTC (2) F: ATGGATATGCACTGCAAAGCAGAC R: TACTAGTGGCGGTCATAGGCAG
c-myc (NM_002467)	Human fetal kidney	(1) F: CCACGAAACTTTGCCCATAGC R: CAGCCAAGGTTGTGAGGTTGC (2) F: ATGCCCTCAACGTTAGCR: TTACGCACAAGAGTTCCGTAG
mycN (NM_005378)	Mouse embryonic kidney	(1) F: AGGAAGCACTCCCCCATATTR: CCACCTCTCATTACCCAGGA (2) F: ATGCCAGCTGCACCGCGTR: TTAGCAAGTCCGAGCGTGTTCG
Oct4 (NM_002701)	Human ESC	(1) F: CCTTCGCAAGCCCTCATTTCTR: CCATTCCTAGAAGGGCAGGC (2) F: ATGGCGGGACACCTGGCTTCR: TCAGTTTGAATGCATGGGAGAGC

Snai1 (NM_005985)	Human fetal kidney	(1) F: GCTACTGCTGCGCGAATCG R: GGTCCTTCCTGCTGGAGCTG (2) F: ATGCCGCGCTCTTTCCTCGTC R: TCAGCGGGGACATCCTGAGCAG
Snai2 (NM_003068)	Human fetal kidney	(1) F: GATGCTGTAGGGACCGCCR: CGAGTAAACATTGATTGCG (2) F: ATGCCGCGCTCCTTCCTG R: TCAGTGTGCTACACAGCAGCC
Hmga2 (NM_003483.4)	Mouse embryonic kidney	(1) F: TCCAACCTCTTCCTCTCCTTCG R: TCAACCACACACCCTTGCTTATC (2) F: ATGAGCGCACGCGGTGAGGGC R: CTAATCCTCCTCTGCGGACTC
Secondary PCR tags	F: GGGGACAACCTTTGTACAAAAAAGTTGGC R: GGGGACAACCTTTGTACAAGAAAGTTGGGTA	

Supplementary Table 2. Forward and reverse primers sequences used for qRT-PCR of endogenous gene expression.

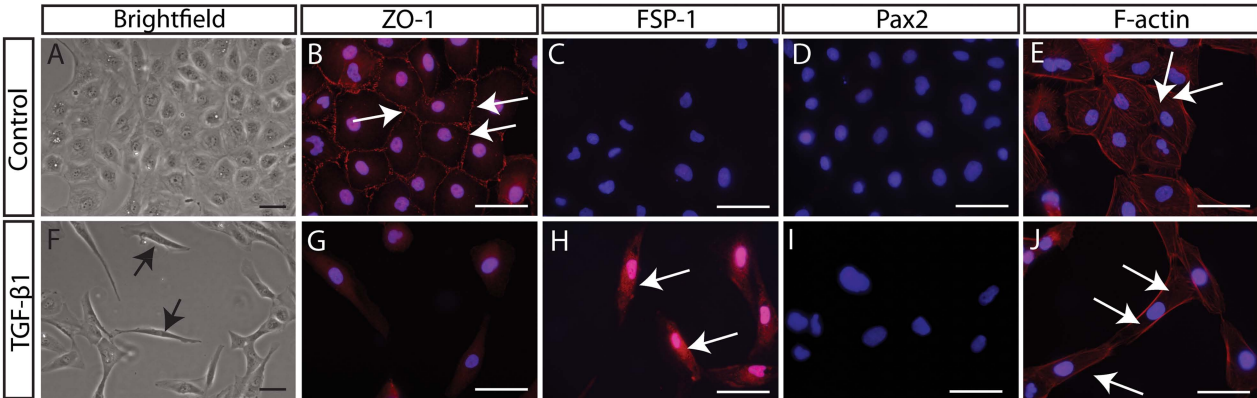
Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	CTCTCTGCTCCTCCTGTTTCGAC	TGAGCGATGTGGCTCGGCT
E-cadherin	CCCGGGACAACGTTTATTAC	GCTGGCTCAAGTCAAAGTCC
MMP2	CGTCGCCCATCATCAAGTTC	GCAGCCATAGAAGGTGTTTCAGG
MMP9	CCAGTCCACCCTTGTGCTCTTC	CCACCCGAGTGTAACCATAGCG
Six2	TCCTGGTCCCTCCGTATGTA	TAGGGGCAGATAGACCACCA
Sall1	GCAAGCGTTAACTTTGGTCTTCTG	CCTAGCAGGGCAACTTGCAA
Pax2	AGGACCAGTTTCCATAGACTGC	GGGAGGGGGCATCAAGTA
Meox2	CTCTGGGACCACCTTCTTTTGG	GCACTTCTGCAGAGCTCGGA
Osr1	ATAGAACCTGTGGGTCACAAGGAC	GGGACAATGTTGGAGAGGTGG
Cited1	AACTTCTGCCAAGGCTCTGA	CACTGCTTTGCGATCTTTCA

Supplementary Table 3. Forward and reverse primer sequences used for RT-PCR.

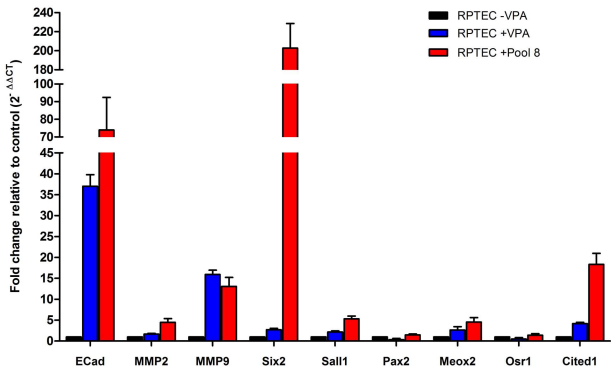
Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	CGAGATCCCTCCAAAATCAA	GTCTTCTGGGTGGCAGTGAT
Six2	CGCCCATGTGGGTCAGTGGG	AGCCGGGAGCGCTGTAGTCA
Cited1	GGTCTGGTTAGAGCGCGTCC	GCAGGCCTCGACGTTGTTGG
Sall1	ACCCCCACCAGTCACGTCCC	TGCCCATGTGTACCTTAAGATTGCC
FoxD1	TGGGGACTCTGCACCAAGGGACT	CGCGCCTGGAGGAGCGAACA
GSC	TCTCAACCAGCTGCACTGTC	TCGTCTGTCTGTGCAAGTCC
FoxA2	GCCATGCACTCGGCTTCCAGTA	CAGCGCCACGTACGACGAC
Pax6	GGCAACCTACGCAAGATGGC	TGAGGGCTGTGTCTGTTCCG
Nanog	GCTTGTCCCCAAAGCTTGCC	TCTTGCATCTGCTGGAGGCTGA

Supplementary Table 4. Antibodies used for immunofluorescence.

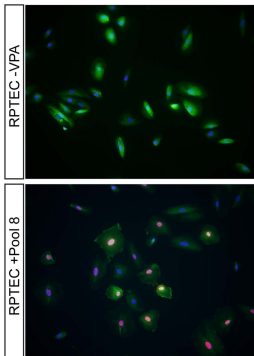
Antigen	Distributor	Catalogue number
Pax2	Zymed Laboratories Inc	71-6000
Wt1	DakoCytomation	6F-H2 m3561
Six2	Sapphire Bioscience	H00010736
Cited1	Sapphire Bioscience	Ab15096
Calbindin	Sigma Aldrich	C9848
Laminin	Sigma Aldrich	L9393
Aquaporin 1	Millipore Australia	AB2219
Ki67	Leica Microsystems	NCL-ki67p
Phosphohistone H3	Millipore Australia	06-570
GFP	Sapphire Bioscience	ab13970
hrGFP	Integrated Science	240142
Mouse IgG	Invitrogen Australia	A11001 (green) and 11005 (red)
Rabbit IgG	Invitrogen Australia	A11008 (green) and 11012 (red)



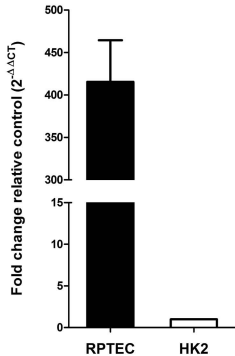
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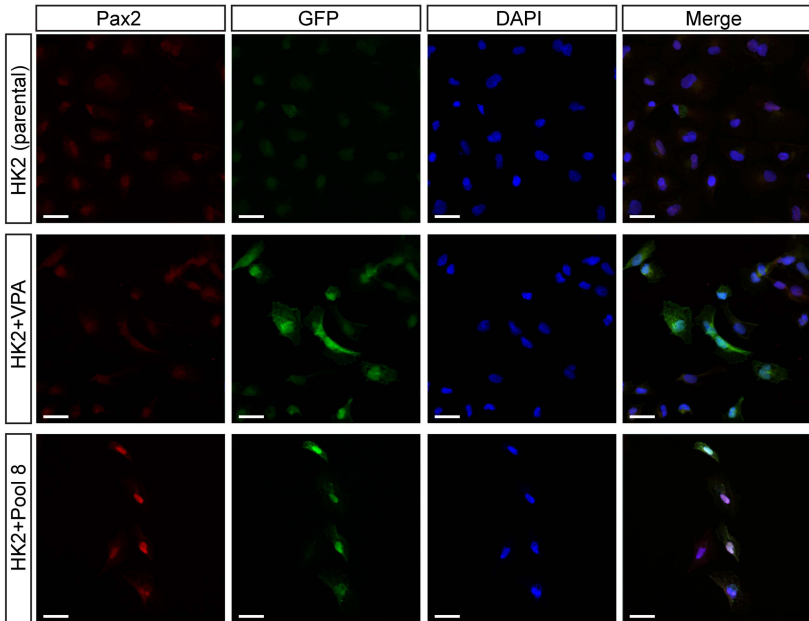


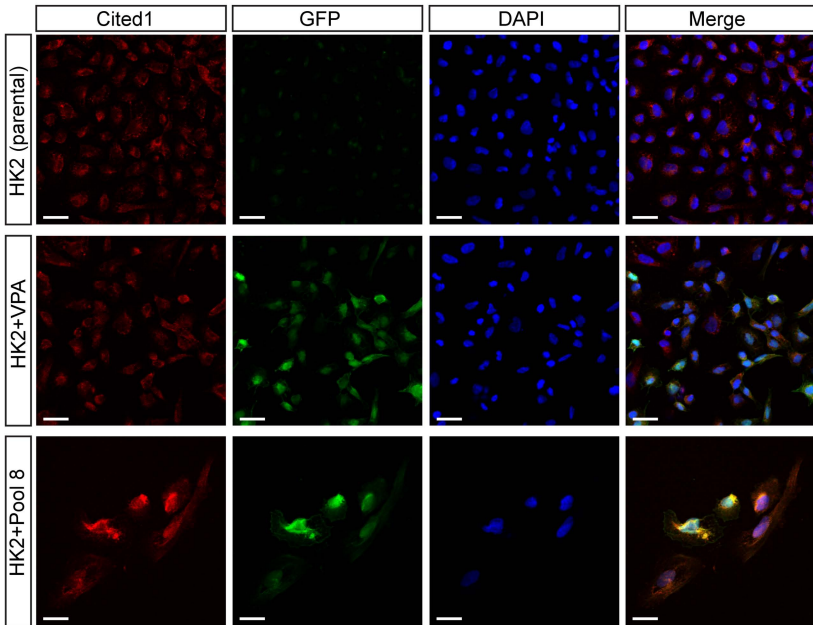
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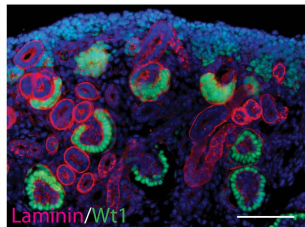
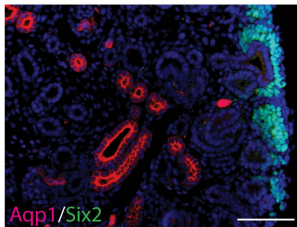
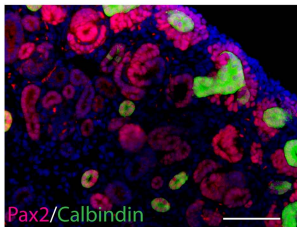
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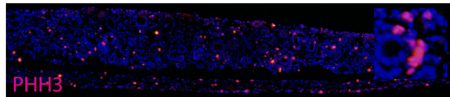
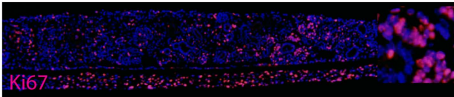
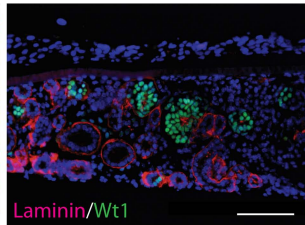
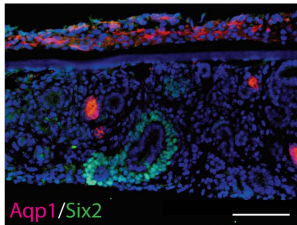
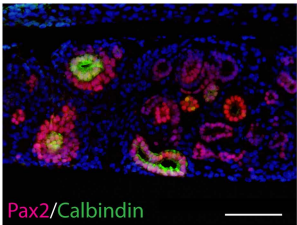


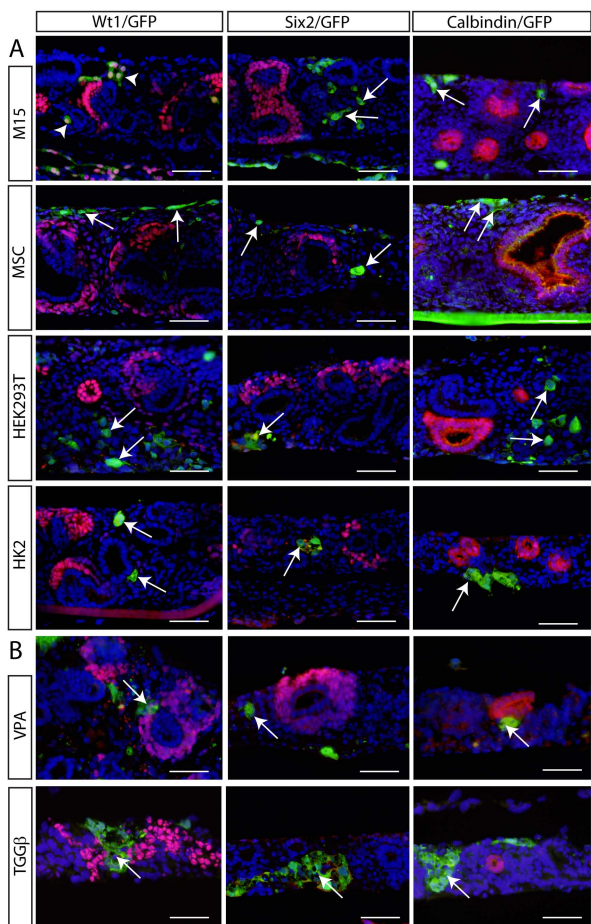


E15.5 kidney



Recombination

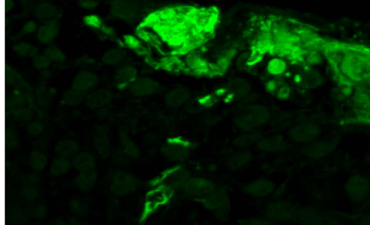




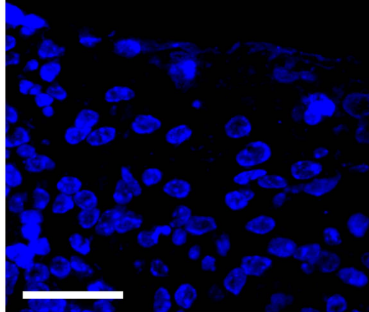
pHH3



GFP



DAPI



Merge

