

Research Article

Hydrocephalus in *Nfix*^{-/-} mice is underpinned by changes in ependymal cell physiology

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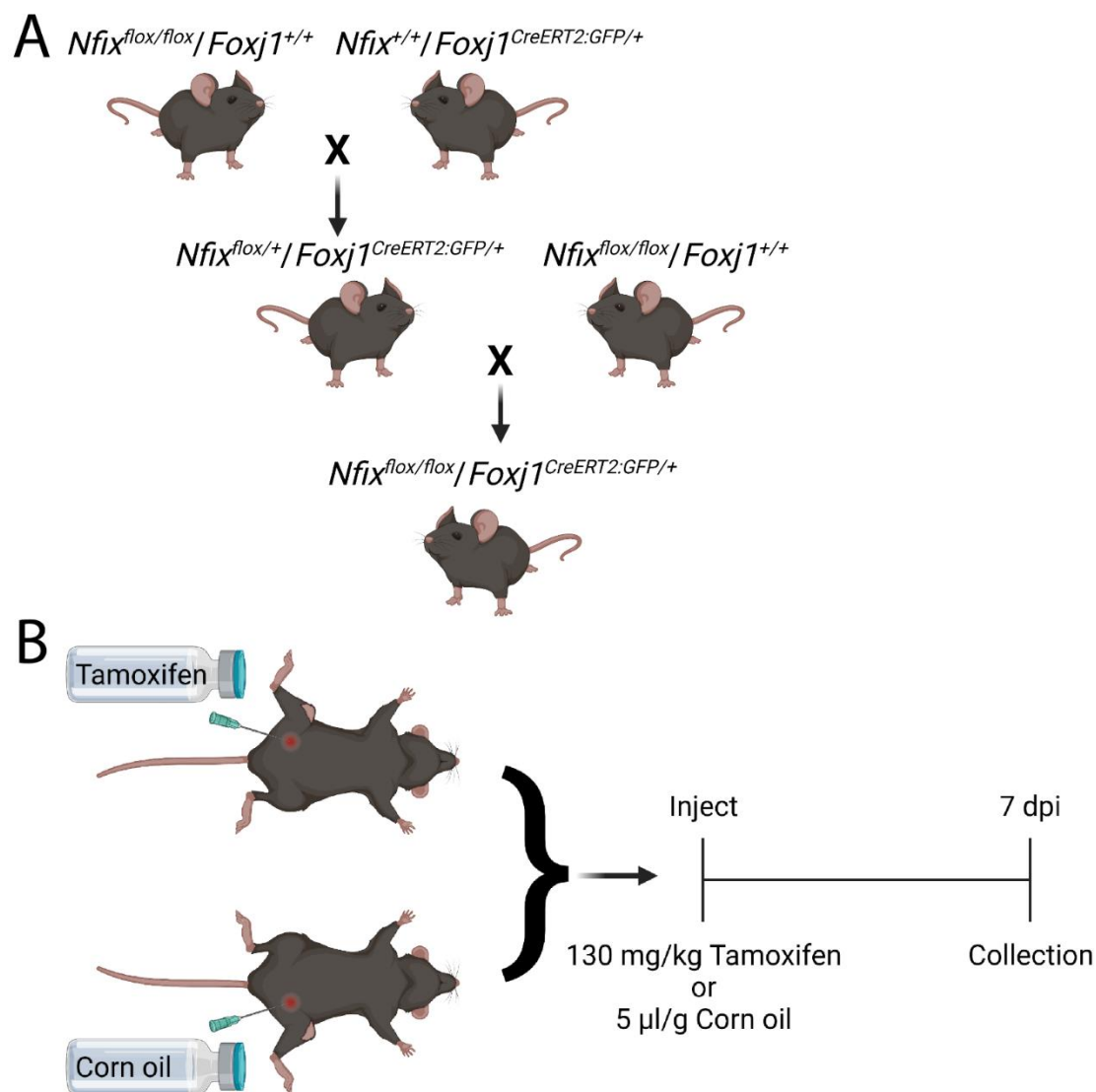


Figure S1. Generation and tamoxifen treatment of the $Nfix^{iFOXJ1-GFP}$ mouse line. **(A)** $Nfix^{+/+};Foxj1^{CreERT2-GFP/+}$ mice were obtained and crossed with $Nfix^{flox/flox};Foxj1^{+/+}$ mice. From the resulting progeny $Nfix^{flox/+};Foxj1^{CreERT2-GFP/+}$ mice were selected and crossed to $Nfix^{flox/flox};Foxj1^{+/+}$ mice. The crossing of these mice produced $Nfix^{flox/flox};Foxj1^{CreERT2-GFP/+}$ mice among other genotypes. These $Nfix^{flox/flox};Foxj1^{CreERT2-GFP/+}$ mice were further crossed with $Nfix^{flox/flox};Foxj1^{+/+}$ mice to maintain the colony or were otherwise used as experimental animals. **(B)** $Nfix^{iFOXJ1-GFP}$ mice were treated with one intraperitoneal injection of 130 mg/kg tamoxifen dissolved in corn oil (experimental) or equivalent volume corn oil at 5 μ l/g (control). Mice were monitored twice daily for signs of stress, changes in body weight or abnormal behaviour. Mice were collected for transcardial perfusion at 7 days post injection (dpi). Figure created with BioRender.com.

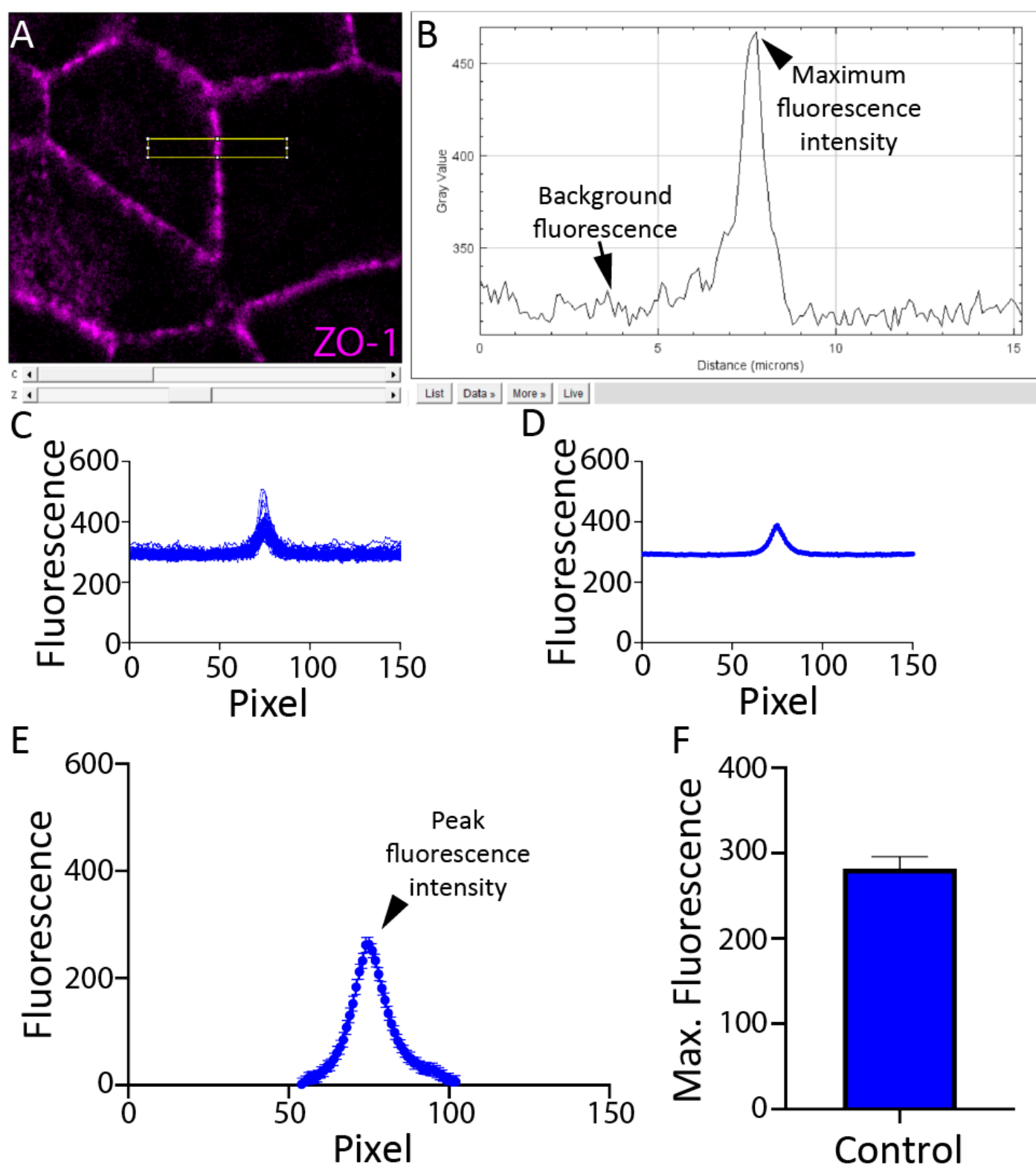


Figure S2. Line scanning analysis protocol. (A) Images of fluorescence stains against adhesion markers are opened in Fiji (ImageJ). Boxes 150 pixels by 20 pixels are drawn perpendicular to and centred on the cell junction (yellow). (B) Using the plot tool, average immunofluorescence is plotted across the cell junction. Black arrow depicts background fluorescence, black arrowhead depicts peak fluorescence. (C) Measurements are repeated 50 times per section along different cell junctions. (D) Average measurement is taken for each section. (E) A background correction is performed by subtracting the median fluorescent intensity from each point. (F) The maximum value from each measurement (maximum fluorescence) are collected and plotted separately. Graph depicts mean \pm s.e.m.

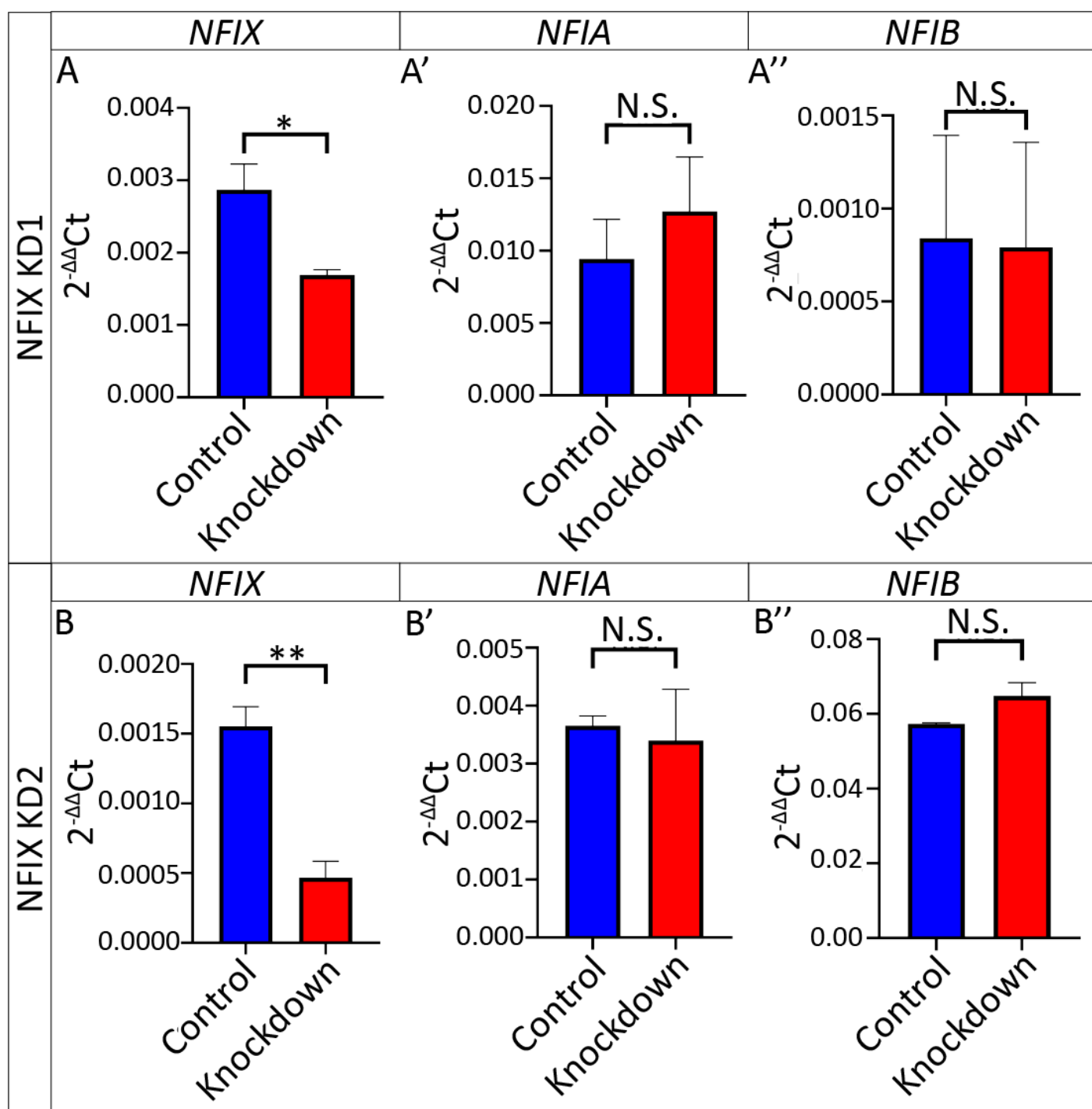


Figure S3. MCF7 cells treated with NFIX knockdown lentivirus demonstrate reduced *NFIX* via qPCR. (A,B) Panels depict $2^{-\Delta\Delta Ct}$ for *NFIX*, *NFIA* and *NFIB* normalised to housekeeping gene *GAPDH* for MCF7 cells treated with two different NFIX knockdown lentivirus constructs (NFIX KD1, A) (NFIX KD2, B) compared to scramble controls. *NFIX* expression in MCF7 cells treated with construct NFIX KD1 (A) and NFIX KD2 (B) demonstrate significant downregulation compared to controls. (A',B') No significant difference was observed in *NFIA* expression between treatment groups. (A'',B'') No significant difference in *NFIB* expression was observed between treatment groups. * $p < 0.05$, ** $p < 0.01$, two-tailed t-test. Graphs depict mean \pm s.e.m. from three samples per genotype.

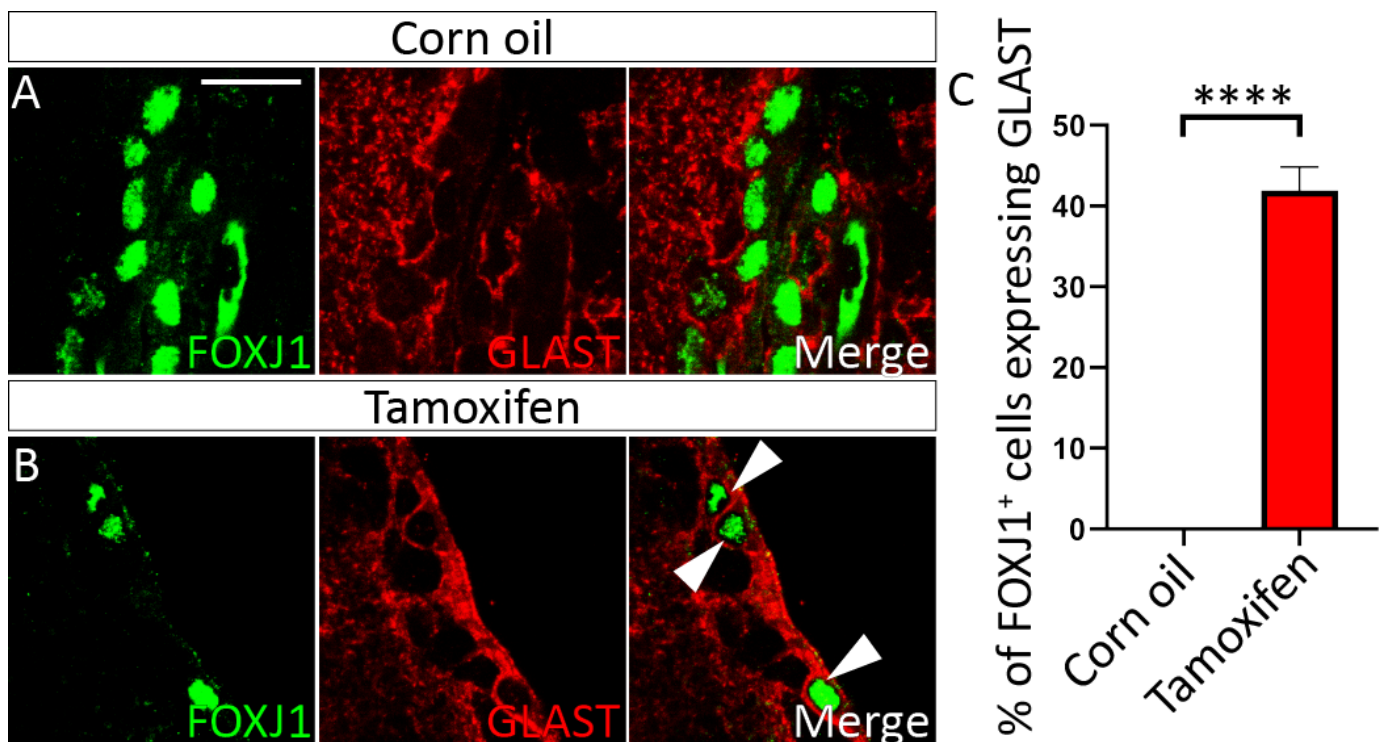


Figure S4. Tamoxifen treated *NfixiFOXJ1-GFP* mice demonstrate FOXJ1 and GLAST co-staining in contrast to wild type controls. (A,B) Lateral ventricle sections of wild type (A) and tamoxifen treated adult *NfixiFOXJ1-GFP* (B) ependyma marked with antibodies against ependymal marker FOXJ1 (green), radial glia marker GLAST (red) and nuclear marker DAPI (grey), displayed as separate images or merged. White arrowheads are used to mark FOXJ1⁺, GLAST⁺ cells. (C) The percentage of FOXJ1⁺ cells that also expressed GLAST is displayed for tamoxifen treated *NfixiFOXJ1-GFP* ependymal cells and controls. The percentage of FOXJ1⁺ cells expressing GLAST is higher in tamoxifen treated *NfixiFOXJ1-GFP* mice compared to controls. **** $p < 0.0001$ two-tailed t-test. Graphs depict mean \pm s.e.m. from three samples per genotype. Scale bar (A) represents 25 μ m.

Table S1. All genes screened via “human adherens junction genes” RT² qPCR array. $2^{-\Delta\Delta Ct}$ scores were calculated relative to *GAPDH* as a normalising housekeeping gene. Genes with a fold change > 2 or < 0.5 have had their fold change marked in bold. Genes with a p-value < 0.05 have had their p-value marked in bold. All calculations were performed using the “RT² qPCR profiler Array Data Analysis Spreadsheet” in excel.

Gene	Fold Change	$2^{-\Delta\Delta Ct}$		p-value
		Knockdown	Scramble	
<i>ACTN1</i>	0.83	1.5E-01	1.8E-01	0.115664
<i>ACTN2</i>	1.06	9.9E-06	9.4E-06	0.906610
<i>ACTN3</i>	2.28	1.0E-04	4.5E-05	0.964520
<i>ACTN4</i>	0.83	3.2E-01	3.8E-01	0.020277
<i>AJAP1</i>	0.87	3.7E-04	4.3E-04	0.576403
<i>ANAPC1</i>	1.15	1.1E-02	9.2E-03	0.110616
<i>ARF6</i>	0.96	2.5E-01	2.7E-01	0.399816
<i>ARVCF</i>	0.80	1.4E-03	1.7E-03	0.082617
<i>BAIAP2</i>	0.59	3.1E-03	5.2E-03	0.159858
<i>CBLL1</i>	1.06	4.2E-02	4.0E-02	0.304813

<i>CDC27</i>	0.99	2.0E-02	2.0E-02	0.867347
<i>CDC42</i>	0.98	1.3E-01	1.4E-01	0.809091
<i>CDH1</i>	1.08	2.8E-01	2.6E-01	0.449571
<i>CDH2</i>	0.68	2.6E-06	3.8E-06	0.199507
<i>CDH3</i>	0.92	5.4E-02	5.8E-02	0.402450
<i>CDH4</i>	1.07	6.6E-05	6.2E-05	0.633885
<i>CDH5</i>	0.75	4.8E-06	6.4E-06	0.669063
<i>CDSN</i>	1.40	1.6E-03	1.2E-03	0.046956
<i>CSNK2A1</i>	0.90	6.5E-02	7.2E-02	0.271377
<i>CSNK2A2</i>	0.77	5.2E-03	6.7E-03	0.043101
<i>CSNK2B</i>	1.15	8.7E-02	7.6E-02	0.119951
<i>CTNNA1</i>	0.94	1.8E-01	1.9E-01	0.496065
<i>CTNNA2</i>	0.68	2.6E-06	3.8E-06	0.199507
<i>CTNNA3</i>	0.46	2.8E-06	6.0E-06	0.074072
<i>CTNNB1</i>	0.94	2.4E-02	2.5E-02	0.608607
<i>CTNND1</i>	0.72	4.5E-02	6.3E-02	0.040615
<i>DLG5</i>	0.90	1.3E-02	1.4E-02	0.245192
<i>DLL1</i>	0.84	4.0E-03	4.7E-03	0.190092
<i>DNM1</i>	0.94	1.1E-02	1.2E-02	0.699427
<i>DNM2</i>	0.90	1.6E-02	1.8E-02	0.536169
<i>DOCK4</i>	0.84	6.0E-05	7.1E-05	0.330424
<i>DSC1</i>	0.81	7.6E-05	9.3E-05	0.466802
<i>DSC2</i>	0.96	9.2E-03	9.6E-03	0.625386
<i>DSC3</i>	0.86	3.3E-06	3.8E-06	0.810542
<i>DSG1</i>	0.32	9.7E-06	3.0E-05	0.235319
<i>DSG2</i>	1.07	4.6E-02	4.4E-02	0.403351
<i>DSG3</i>	0.48	3.0E-06	6.2E-06	0.373559
<i>DSG4</i>	1.82	6.9E-06	3.8E-06	0.281453
<i>DSP</i>	1.16	1.2E-01	1.1E-01	0.029349
<i>EXOC2</i>	0.88	1.5E-02	1.7E-02	0.077498
<i>FARP2</i>	0.88	1.2E-03	1.4E-03	0.187330
<i>FLNA</i>	0.94	3.7E-01	4.0E-01	0.566237
<i>FLNB</i>	0.83	1.7E-01	2.1E-01	0.320782
<i>HGS</i>	1.02	6.9E-03	6.7E-03	0.710078
<i>IQGAP1</i>	1.01	9.4E-02	9.4E-02	0.931711
<i>JUP</i>	0.83	6.6E-02	8.0E-02	0.125040
<i>LMO7</i>	0.87	4.2E-03	4.8E-03	0.230840
<i>MAPRE1</i>	1.00	9.2E-02	9.2E-02	0.952960
<i>MAPRE2</i>	0.89	7.8E-04	8.8E-04	0.282487
<i>MLLT4</i>	0.88	1.2E-02	1.4E-02	0.302713
<i>NME1</i>	1.31	1.4E-01	1.1E-01	0.005504

<i>NOTCH1</i>	0.94	5.0E-03	5.3E-03	0.583941
<i>NOTCH2</i>	1.07	4.2E-02	3.9E-02	0.437137
<i>NOTCH3</i>	0.74	2.5E-02	3.4E-02	0.136389
<i>NOTCH4</i>	0.85	2.4E-04	2.8E-04	0.313678
<i>P2RX6</i>	0.62	2.5E-05	4.1E-05	0.404917
<i>PARD3</i>	0.98	1.1E-02	1.1E-02	0.711133
<i>PERP</i>	1.23	1.4E-03	1.1E-03	0.141632
<i>PIK3CG</i>	0.68	2.6E-06	3.8E-06	0.199507
<i>PKP1</i>	0.91	1.4E-02	1.6E-02	0.301747
<i>PKP2</i>	1.19	1.7E-02	1.4E-02	0.043531
<i>PKP3</i>	0.96	4.6E-02	4.8E-02	0.764469
<i>PKP4</i>	0.87	4.6E-02	5.4E-02	0.061466
<i>PNN</i>	1.18	5.9E-02	5.0E-02	0.237154
<i>PPAP2B</i>	0.84	7.1E-03	8.4E-03	0.292160
<i>PPL</i>	0.99	5.9E-02	6.0E-02	0.962484
<i>PVRL1</i>	0.88	2.6E-02	2.9E-02	0.120517
<i>PVRL2</i>	0.89	1.3E-01	1.4E-01	0.350571
<i>PVRL3</i>	1.23	1.7E-03	1.4E-03	0.220280
<i>PVRL4</i>	0.74	1.2E-02	1.7E-02	0.153740
<i>RAC1</i>	1.01	2.3E-01	2.3E-01	0.806500
<i>RALA</i>	0.88	6.4E-02	7.3E-02	0.294289
<i>RHOA</i>	0.95	1.0E-01	1.0E-01	0.422377
<i>SORBS1</i>	1.13	7.2E-04	6.4E-04	0.263173
<i>SSX2IP</i>	0.92	1.1E-02	1.2E-02	0.438691
<i>TJP1</i>	1.13	6.8E-02	6.0E-02	0.019167
<i>TLN1</i>	0.90	1.6E-02	1.7E-02	0.504728
<i>TLN2</i>	1.04	2.8E-03	2.7E-03	0.730490
<i>VCL</i>	0.82	4.8E-02	5.8E-02	0.055765
<i>VEZT</i>	1.07	2.2E-02	2.1E-02	0.214859
<i>WAS</i>	0.94	3.4E-03	3.6E-03	0.658110
<i>WASF1</i>	1.16	8.7E-03	7.5E-03	0.003203
<i>WASL</i>	0.88	7.7E-03	8.8E-03	0.341148
<i>ZYX</i>	0.73	8.1E-02	1.1E-01	0.061866
<i>ACTB</i>	0.80	5.6E+00	6.9E+00	0.177284
<i>B2M</i>	0.90	4.9E-01	5.5E-01	0.056493
<i>GAPDH</i>	1.15	1.7E+00	1.5E+00	0.063450
<i>HPRT1</i>	1.18	6.3E-02	5.3E-02	0.057648
<i>RPLP0</i>	1.02	3.4E+00	3.3E+00	0.835443

Table S2. All genes screened via “human extracellular matrix and adhesion molecules” RT² qPCR array. $2^{-\Delta\Delta C_t}$ scores were calculated relative to *GAPDH* as a normalising housekeeping gene. Genes with a fold change > 2 or < 0.5 have had their fold change marked in bold. Genes with a p-value < 0.05 have had their p-value marked in bold. All calculations were performed using the “RT² qPCR profiler Array Data Analysis Spreadsheet” in excel.

Gene	$2^{-\Delta\Delta C_t}$			
	Fold Change	Knockdown	Scramble	p-value
<i>ADAMTS1</i>	1.77	4.5E-05	2.5E-05	0.610378
<i>ADAMTS13</i>	0.59	1.7E-03	2.8E-03	0.096113
<i>ADAMTS8</i>	0.53	1.2E-05	2.3E-05	0.492762
<i>CD44</i>	0.58	9.5E-02	1.6E-01	0.013010
<i>CDH1</i>	1.09	5.3E-01	4.9E-01	0.622432
<i>CLEC3B</i>	0.57	1.1E-05	2.0E-05	0.395492
<i>CNTN1</i>	0.75	5.1E-06	6.8E-06	0.567471
<i>COL11A1</i>	2.14	1.5E-05	6.8E-06	0.207364
<i>COL12A1</i>	0.99	1.7E-02	1.7E-02	0.843418
<i>COL14A1</i>	0.29	9.8E-05	3.4E-04	0.394398
<i>COL15A1</i>	1.21	8.2E-06	6.8E-06	0.530113
<i>COL16A1</i>	0.61	5.1E-04	8.5E-04	0.187912
<i>COL1A1</i>	0.97	4.1E-03	4.2E-03	0.938625
<i>COL4A2</i>	1.98	4.6E-04	2.3E-04	0.182559
<i>COL5A1</i>	1.30	4.0E-02	3.1E-02	0.640309
<i>COL6A1</i>	0.59	5.2E-03	8.9E-03	0.005121
<i>COL6A2</i>	0.68	2.1E-03	3.1E-03	0.335268
<i>COL7A1</i>	0.73	4.9E-04	6.7E-04	0.097175
<i>COL8A1</i>	0.58	5.1E-06	8.8E-06	0.301919
<i>CTGF</i>	1.64	2.1E-03	1.3E-03	0.517101
<i>CTNNA1</i>	0.92	2.7E-01	3.0E-01	0.759017
<i>CTNNB1</i>	0.92	3.6E-02	3.9E-02	0.561418
<i>CTNND1</i>	0.80	9.0E-02	1.1E-01	0.106207
<i>CTNND2</i>	1.07	8.0E-03	7.5E-03	0.953266
<i>ECM1</i>	1.07	2.1E-04	2.0E-04	0.999658
<i>FN1</i>	0.68	3.2E-02	4.6E-02	0.182809
<i>HAS1</i>	0.73	6.5E-05	8.9E-05	0.543897
<i>ICAM1</i>	0.54	3.8E-03	7.1E-03	0.009810
<i>ITGA1</i>	1.05	1.9E-04	1.8E-04	0.598848
<i>ITGA2</i>	1.12	2.2E-02	1.9E-02	0.562210
<i>ITGA3</i>	0.71	4.4E-02	6.2E-02	0.023212
<i>ITGA4</i>	0.16	1.1E-05	6.9E-05	0.033471
<i>ITGA5</i>	0.75	7.0E-03	9.3E-03	0.430683
<i>ITGA6</i>	1.38	2.4E-03	1.7E-03	0.126874

<i>ITGA7</i>	0.69	5.9E-04	8.6E-04	0.242675
<i>ITGA8</i>	0.53	5.1E-06	9.7E-06	0.234502
<i>ITGAL</i>	1.15	1.5E-04	1.3E-04	0.641042
<i>ITGAM</i>	0.86	5.0E-04	5.8E-04	0.582762
<i>ITGAV</i>	0.67	7.3E-02	1.1E-01	0.138623
<i>ITGB1</i>	0.82	2.0E-01	2.4E-01	0.033797
<i>ITGB2</i>	0.85	1.6E-03	1.8E-03	0.696834
<i>ITGB3</i>	1.27	4.1E-05	3.2E-05	0.781702
<i>ITGB4</i>	1.12	5.9E-02	5.2E-02	0.535322
<i>ITGB5</i>	0.90	5.2E-02	5.7E-02	0.517014
<i>ANOS1</i>	1.58	3.6E-04	2.3E-04	0.356454
<i>LAMA1</i>	0.72	5.1E-06	7.2E-06	0.483307
<i>LAMA2</i>	0.49	7.1E-05	1.5E-04	0.387150
<i>LAMA3</i>	0.75	4.9E-03	6.6E-03	0.394555
<i>LAMB1</i>	1.18	1.7E-03	1.4E-03	0.780743
<i>LAMB3</i>	0.41	2.6E-02	6.3E-02	0.139120
<i>LAMC1</i>	0.77	7.5E-02	9.7E-02	0.030582
<i>MMP1</i>	0.60	7.9E-06	1.3E-05	0.732275
<i>MMP10</i>	0.82	7.3E-06	8.9E-06	0.396895
<i>MMP11</i>	1.02	1.1E-03	1.1E-03	0.815091
<i>MMP12</i>	0.34	6.6E-04	2.0E-03	0.599603
<i>MMP13</i>	0.75	5.1E-06	6.8E-06	0.567471
<i>MMP14</i>	0.41	1.4E-04	3.6E-04	0.054865
<i>MMP15</i>	1.06	2.1E-04	2.0E-04	0.884209
<i>MMP16</i>	0.84	3.2E-03	3.8E-03	0.597330
<i>MMP2</i>	1.41	1.5E-05	1.0E-05	0.578603
<i>MMP3</i>	0.86	5.9E-06	6.8E-06	0.779029
<i>MMP7</i>	0.58	8.2E-06	1.4E-05	0.403380
<i>MMP8</i>	0.57	9.9E-06	1.8E-05	0.201549
<i>MMP9</i>	1.09	2.1E-03	1.9E-03	0.440664
<i>NCAM1</i>	0.77	3.3E-05	4.2E-05	0.666247
<i>PECAM1</i>	1.31	6.8E-05	5.2E-05	0.883502
<i>SELE</i>	0.85	1.1E-05	1.3E-05	0.804218
<i>SELL</i>	0.60	3.9E-03	6.5E-03	0.019988
<i>SELP</i>	1.91	1.3E-05	6.8E-06	0.151765
<i>SGCE</i>	0.82	4.2E-03	5.1E-03	0.144015
<i>SPARC</i>	1.14	6.8E-05	5.9E-05	0.559416
<i>SPG7</i>	0.83	3.7E-02	4.5E-02	0.149686
<i>SPP1</i>	0.48	5.1E-06	1.1E-05	0.251592
<i>TGFBI</i>	0.55	2.4E-02	4.3E-02	0.310831
<i>THBS1</i>	1.19	1.4E-01	1.2E-01	0.271260

<i>THBS2</i>	0.15	2.2E-05	1.4E-04	0.002481
<i>THBS3</i>	0.60	6.3E-03	1.1E-02	0.088154
<i>TIMP1</i>	0.81	1.9E-02	2.3E-02	0.107068
<i>TIMP2</i>	0.81	5.2E-02	6.4E-02	0.497353
<i>TIMP3</i>	0.44	5.9E-03	1.3E-02	0.024631
<i>TNC</i>	3.36	5.1E-05	1.5E-05	0.376510
<i>VCAM1</i>	0.75	5.1E-06	6.8E-06	0.567471
<i>VCAN</i>	0.75	5.1E-06	6.8E-06	0.567471
<i>VTN</i>	1.35	3.7E-04	2.7E-04	0.821234
<i>ACTB</i>	1.03	5.8E+00	5.6E+00	0.920727
<i>B2M</i>	0.82	4.0E-01	4.8E-01	0.526361
<i>GAPDH</i>	1.08	1.8E+00	1.7E+00	0.654743
<i>HPRT1</i>	1.16	6.5E-02	5.6E-02	0.120095
<i>RPLP0</i>	0.95	3.7E+00	3.9E+00	0.797525
