

Using an *in vivo* syngeneic spontaneous metastasis model identifies ID2 as a promoter of breast cancer colonisation in the brain

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Additional file 1; Supplementary Tables

Table S1. MISSION® shRNA pLKO-puro transduction particles

Table S2. ORF Clone Expression Systems (Genecopoeia)

Table S3. Taqman RT-qPCR gene expression assays

Table S4. Antibodies for immunoblotting (IB) and immunohistochemistry (IHC)

Additional file 1; Supplementary Figure

Figure S1. Higher power image of Figure 1c

Figure S2. Heat map of shortlisted genes

Table S1 MISSION shRNA pLKO-puro transduction particles

Clone I.D.	Gene targeted	Species targeted	Named
SHC202V	None	None	shNTC
TRCN0000054390	<i>Id2</i>	Mm	shId2
TRCN0000042078	<i>Aldh3a1</i>	Mm	shAldh3a1

Table S2 ORF clone expression systems

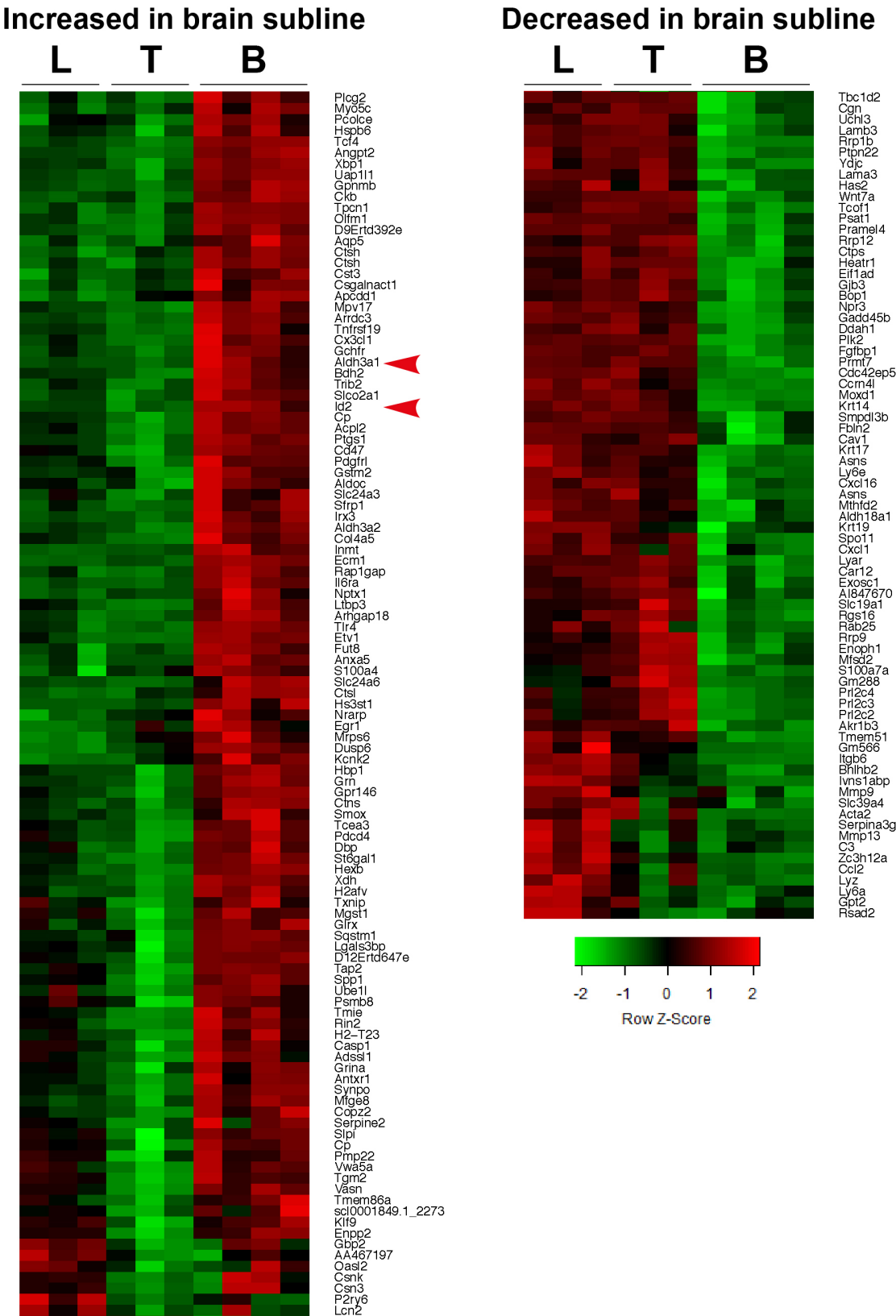
Vector	I.D.	Tag	Species	Gene	Named
pReceiver-LV166	N/A	IRES2-mcherry	N/A		Vec
pReceiver-LV166	Mm03201	IRES2-mcherry	Mouse	Id2 (NM_010496.3)	Id2 OE
pReceiver-LV166	Mm28326	IRES2-mcherry	Mouse	Aldh3a1 (NM_001112725.1)	Aldh3a1 OE

Table S3 Taqman RT-qPCR gene expression assays

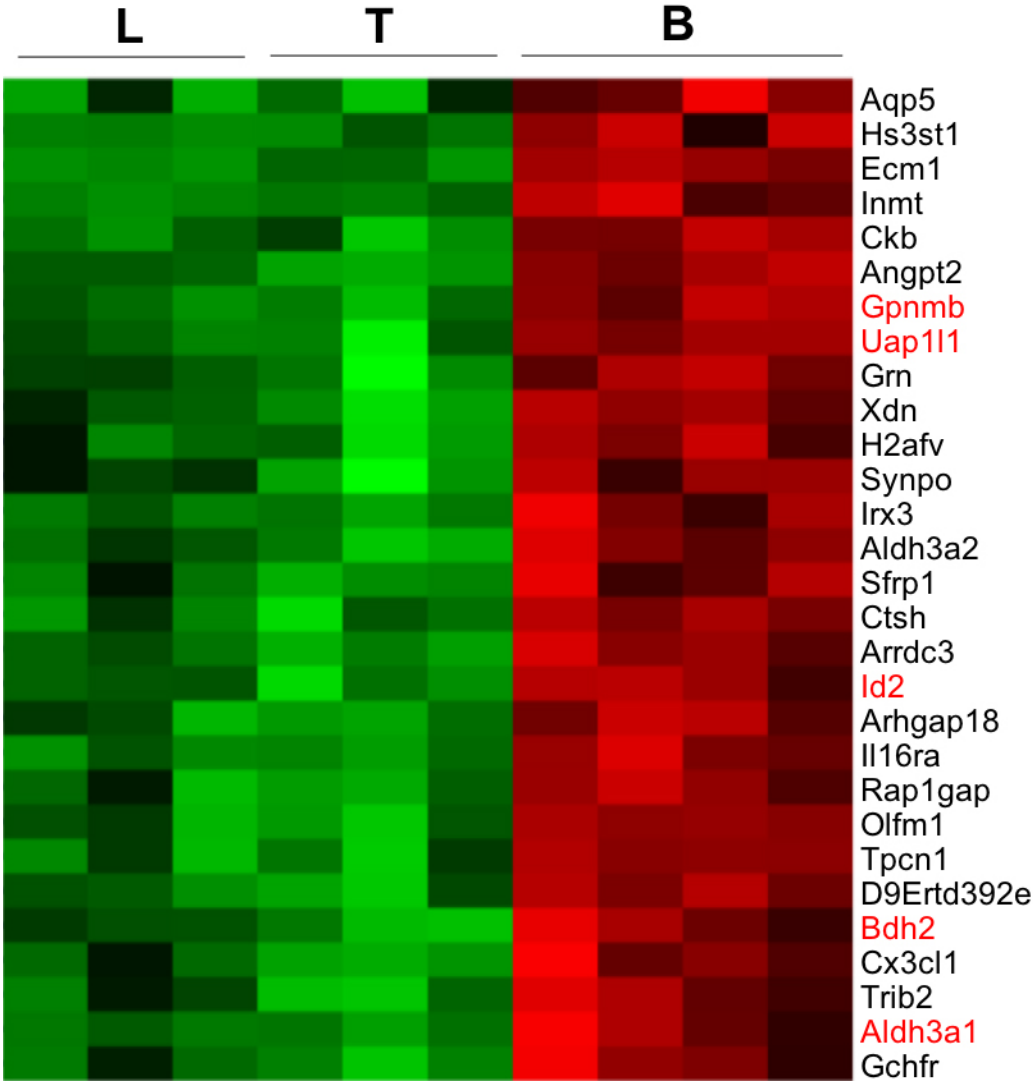
Gene symbol	Gene name	Probe set I.D.	Species
<i>B2m</i>	β -2 microglobulin	Mm00437762_m1	Mm
<i>B2M</i>	β -2 microglobulin	Hs99999907_m1	Hs
<i>Gapdh</i>	Glyceraldehyde-3-phosphate dehydrogenase	4352339E	Mm
<i>Id2</i>	inhibitor of DNA binding 2	Mm00711781_m1	Mm
<i>ID2</i>	inhibitor of DNA binding 2	Hs04187239_m1	Hs
<i>Aldh3a1</i>	aldehyde dehydrogenase 3 family member A1	Mm00839312_m1	Mm
<i>ALDH3A1</i>	aldehyde dehydrogenase 3 family member A1	Hs00964880_m1	Hs
<i>Id1</i>	Inhibitor of DNA binding 1	Mm00775963_g1	Mm
<i>Id3</i>	Inhibitor of DNA binding 3	Mm00492575_m1	Mm
<i>Id4</i>	Inhibitor of DNA binding 4	Mm00499701_m1	Mm

Table S4 Antibodies for immunoblotting (IB) and Immunohistochemistry (IHC)

Antibody target	Host	Supplier	Dilution	Application
Vinculin	Mouse	Cell signalling	1:1000	IB
Id2	Mouse	D39E8 Cell Signalling	1:1000	IB
Aldh3a1	Goat	ARG65260 arigobio	1:10,000	IB
Lamin A/C	Rabbit	Santa Cruz Biotech (sc-2004)	1:500	IHC
CD31	Rat	Dianova (DIA310)	1:75	IHC
Endomucin	Rat	Santa Cruz Biotech (V.7C7; sc- 65495)	1:1000	IHC



SFig. 1 Higher power image of Fig. 1c. Heat map (Pearson, ward.D2) of 186 genes (with official mouse gene symbol) differentially expressed between B and T, between B and L, or between L and T sublines with an absolute fold change ≥ 2.0 , $p < 0.001$. Sublines are in the same order as in Fig. 1a. Arrowheads indicate *Id2* and *Aldh3a1*.



SFig. 2. Heat map of shortlisted genes. Heat map (Pearson, ward.D2) of 29 genes (with official mouse gene symbol) with upregulated expression (absolute fold change ≥ 2.0 , $p < 0.001$) in B versus T and B versus L but not between L and T. Genes further validated by RT-qPCR are shown in red.