

SUPPLEMENTARY FILES

Journal: Gastric Cancer

SFRP4 drives invasion in gastric cancer and is an early predictor of recurrence

Rita A. Busuttil^{1,2,3}, Joshy George⁴, Colin M. House¹, Stephen Lade⁵, Catherine Mitchell⁵, Natasha S. Di Costanzo¹, Sharon Pattison⁶, Yu-Kuan Huang^{1,2,3}, Patrick Tan^{7,8}, Jae-Ho Cheong^{9,10}, Sun Young Rha¹¹ & Alex Boussioutas^{1,2,3}

¹ Upper Gastrointestinal Translational Research Laboratory, Peter MacCallum Cancer Centre, Parkville, Victoria, Australia

² Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia

³ Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia

⁴ Computational Sciences, Jackson Laboratory for Genomic Medicine, Farmington, Connecticut USA

⁵ Department of Pathology, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia

⁶ Department of Medicine, University of Otago, Dunedin, New Zealand

⁷ Genome Institute of Singapore, Singapore.

⁸ Cancer Science Institute of Singapore, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

⁹ Department of Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea

¹⁰ Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, Republic of Korea

¹¹ Division of Medical Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Republic of Korea.

Corresponding author info: Professor Alex Boussioutas, Peter MacCallum Cancer Centre, 305 Grattan St, Parkville Telephone: +61 3 98559 7031, Fax: +61 3 8559 5489, Email: alex.boussioutas@petermac.org

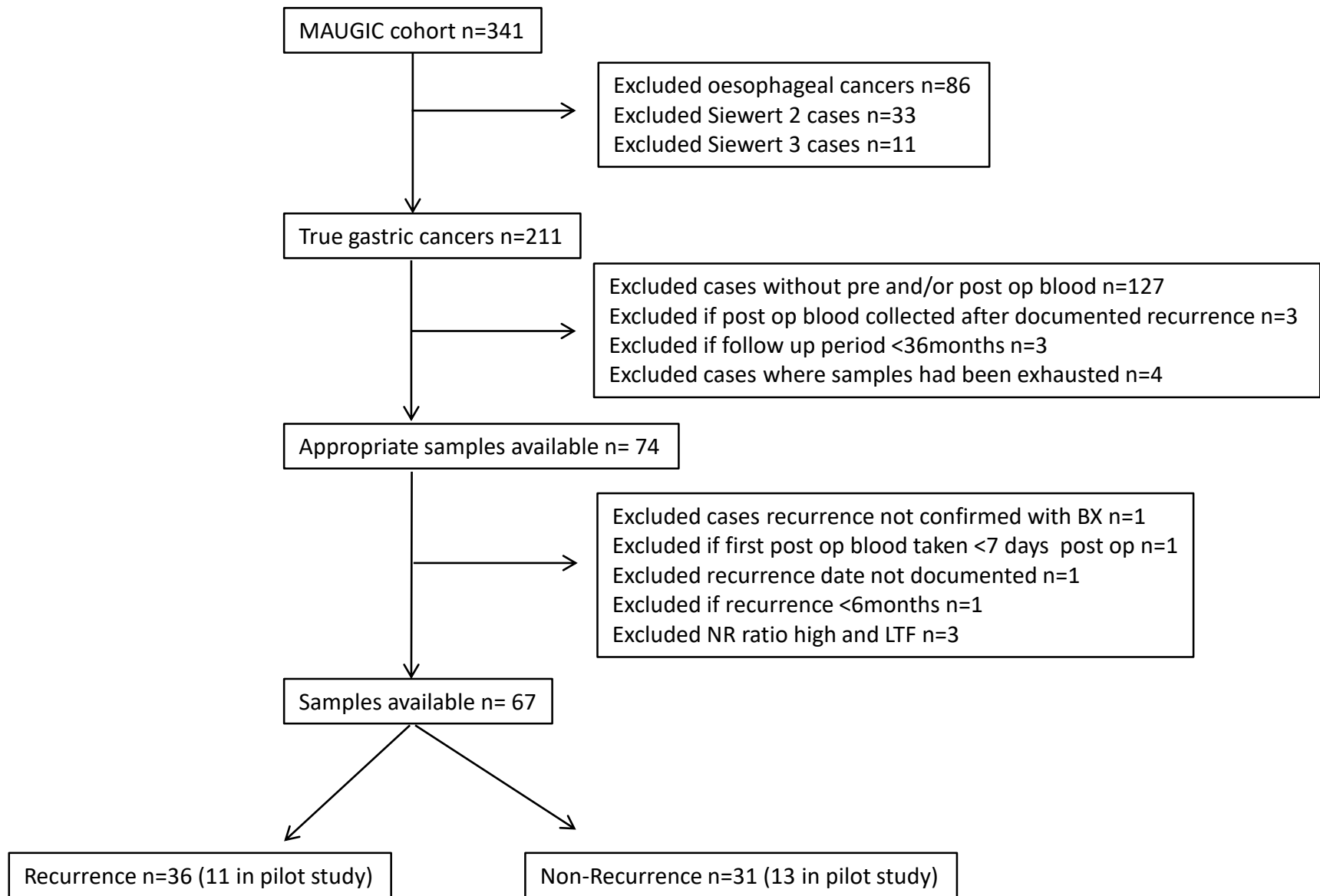


Figure S1. Consort diagram for the selection of gastric cancer plasma samples for SFRP4 ELISA.

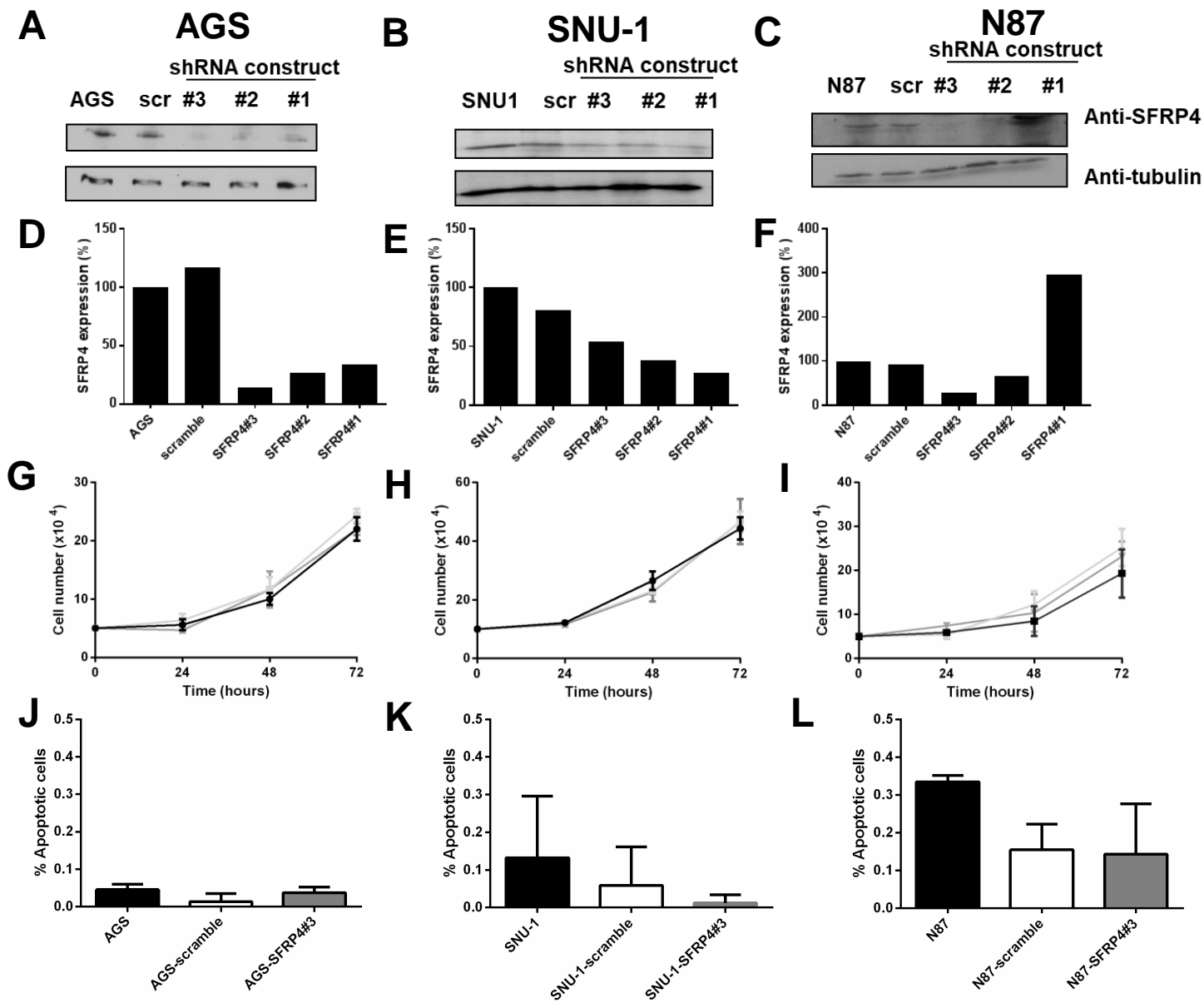


Figure S2. Effects of shRNA mediated knockdown of SFRP4. shRNA mediated knockdown of gastric cancer cell lines (A) AGS, (B) SNU-1 and (C) NCI-N87 was performed using the three available constructs targeting the SFRP4 gene (Supplementary Table S2) and a scramble control. Western blots were performed to confirm knockdown and the resulting immunoblots in (A-C) were quantitated by densitometric methods (D-F) using Image J. Overall construct #3 was the best performing and used for all subsequent experiments. Proliferation was determined by cell counts at 24, 48 and 72 hours (G-I) cell lines. No significant differences were observed in any of the cell lines. (J-L) Apoptosis was determined for the same cell lines using the Apoptaq kit. Representative images are shown in Fig. S3. Data are represented as mean \pm SD.

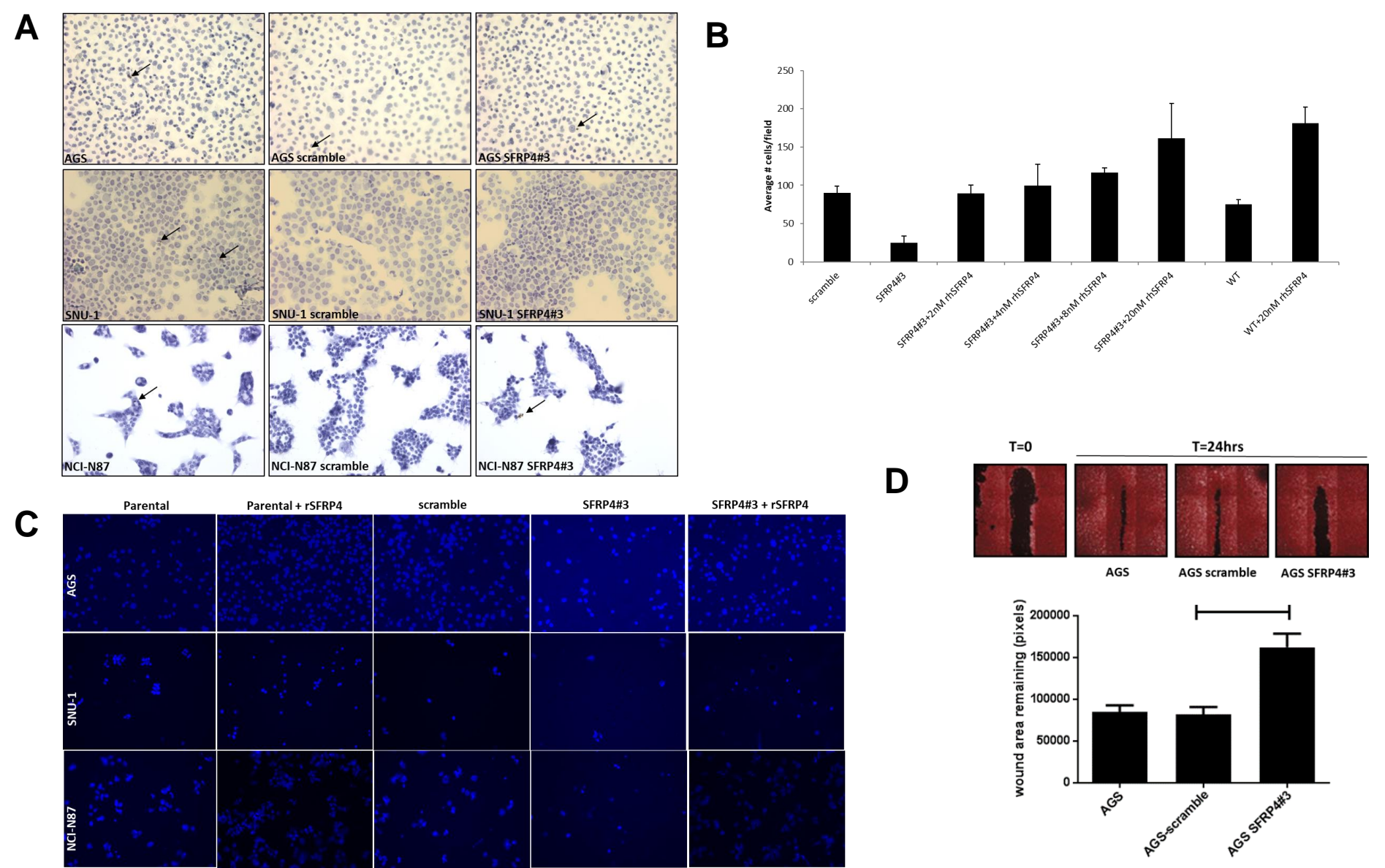
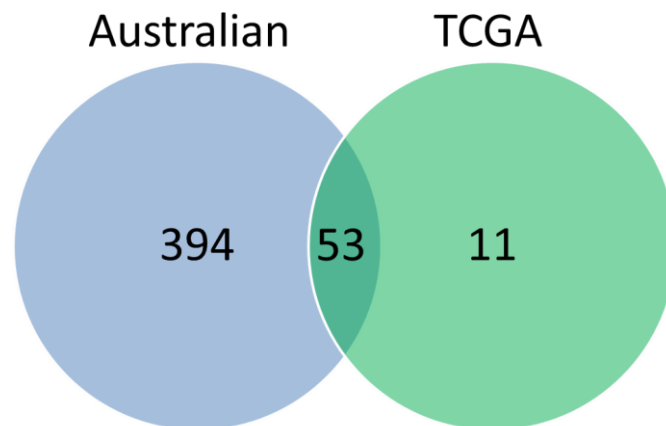


Figure S3. Outcomes of functional assays **(A)** representative images from apoptosis assays (arrows represent apoptotic cells), **(B)** the effect of the addition of recombinant human SFRP4 on the invasion of AGS cells. AGS WT or AGS-SFRP4 knockdown cells were pre-incubated with varying doses of rhSFRP4 before use in an invasion assay. Data are represented as mean \pm SD, **(C)** representative images of AGS, SNU-1 and NCI-N87 cells from invasion assays shown in Fig 3. **(D)** AGS wound healing assay. AGS WT, scramble and SFRP4 knockdown cells were seeded to confluency, scratched to wound and imaged at 0- and 24-hours. Wound healing was then quantitated * $p=6.8 \times 10^{-3}$; t test. Data are represented as mean \pm SD.

Table S2: Genes correlated with SFRN4

Australian dataset										TCGA dataset										Common genes	
Gene	Pearson Score	Gene	Pearson Score	Gene	Pearson Score	Gene	Pearson Score	Gene	Pearson Score	Gene	Pearson Score	Gene	Pearson Score	Gene Symbol	Pearson Score	Gene Symbol	Pearson Score				
ACTA2	0.71	CNN1	0.60	FAM20C	0.72	ISM1	0.70	M8AS	0.76	PROS1	0.65	STON1	0.78	ADGRA2	0.61	ADGRA2	0.61				
ACTN1	0.72	CNRP1	0.65	FAM229B	0.62	ITGA1	0.60	MRC2	0.78	PRRX1	0.79	SULF1	0.76	AEBP1	0.67	AEBP1	0.67				
ADAMTS2	0.62	COL10A1	0.73	FAP	0.81	ITGB5	0.68	MGRPRF	0.69	PRSS23	0.65	SVIL	0.67	ANTXR1	0.69	ANTXR1	0.69				
ADAMT1	0.64	COL12A1	0.61	FBLN1	0.68	ITGBL1	0.90	MVRL1	0.62	PTGFR	0.62	SYNC	0.74	ASPN	0.66	ASPN	0.66				
ADGRA2	0.63	COL14A1	0.64	FBLN2	0.75	JAM3	0.73	MSC-AS1	0.71	PTGIS	0.71	SYNDIG1	0.60	AZIN2	0.63	BGN	0.63				
ADGRD1	0.65	COL16A1	0.65	FBN1	0.84	JAZF1	0.60	M8R83	0.77	PTGL	0.65	SFT11	0.68	BGN	0.68	BK1C1	0.67				
AEBP1	0.63	COL1A1	0.60	FBLX7	0.68	KANK2	0.63	M8R47	0.76	QKI	0.69	TAGLN	0.72	BK1C1	0.62	C1R	0.62				
AKAP12	0.82	COL3A1	0.75	F8X032	0.71	KATNAL1	0.65	M8R48	0.80	RAB23	0.72	TBCEL	0.61	C1QTNF5	0.61	C1S	0.61				
AKT3	0.73	COL5A1	0.72	FERM1T2	0.74	KCNK4	0.64	MVH10	0.79	RAB31	0.81	TECAL7	0.63	C1R	0.67	CDH11	0.78				
ANKOTL1	0.74	COL5A2	0.67	FZT1	0.67	KCNB9	0.63	MPL9	0.68	RAH4	0.68	TC4	0.72	C1S	0.64	CNTN3	0.64				
ANGPT12	0.73	COL6A1	0.61	FGF7F3	0.63	KCTD10	0.68	MPLK	0.70	RARRB2	0.71	TENM4	0.70	CDH11	0.78	COL8A1	0.78				
ANKRD10	0.61	COL6A2	0.78	FGFR1	0.72	KSLCC1	0.67	KSLCN	0.66	RGLI2	0.61	TGFB11	0.71	CNTN3	0.7	CTHRC1	0.7				
ANGSI	0.77	COL6A3	0.64	FBN	0.66	KAA1755	0.65	NAP1L3	0.72	RASFR8	0.74	TGFR3	0.64	COL8A1	0.73	CDAC1	0.73				
ANTXR1	0.89	COL8A1	0.92	FRBP14	0.60	KDIN5220	0.63	NAV3	0.63	RASSF8-AS1	0.60	TGFR1	0.66	CFZ	0.67	DOK5	0.67				
ANKA6	0.66	COL8A2	0.85	FRBP7	0.73	KF1B	0.64	NDN	0.72	RBFOS1	0.64	THBS2	0.87	CTHRC1	0.62	ECM2	0.62				
ADC3	0.63	COLLEC12	0.66	FN1A	0.66	KORREL1	0.74	NEXN	0.67	RBM51	0.66	THBS3	0.75	DAC11	0.66	EFEMP2	0.66				
AP152	0.67	COMP	0.66	FMO1	0.60	KLC1	0.68	ND2	0.64	RBM53	0.67	THBS4	0.73	DOX5	0.63	ELN	0.63				
ARL10	0.63	COPB8	0.61	FN1	0.72	KLHL29	0.66	NSCH	0.61	RCBTB2	0.61	THY1	0.78	ECM2	0.61	FAM19A5	0.61				
ARMCK1	0.68	COF22	0.80	FND1C1	0.82	LAMMA	0.68	NMMT	0.61	RECK	0.65	TIMP1	0.75	EFEMP2	0.6	FAP	0.6				
ARMCK2	0.61	COX7A1	0.69	FOXF2	0.61	LAMB2	0.68	NOTCH2	0.71	RERG	0.67	TIMP2	0.83	ELN	0.68	FBN1	0.68				
ASPN	0.84	CPXM2	0.78	FRMO6	0.72	LAMC1	0.65	NOX4	0.65	RFTN1	0.66	TIMP3	0.73	FAM19A5	0.62	FND1C1	0.62				
ATL3	0.64	CRPRLD1	0.81	FSTL1	0.84	LAPP6	0.64	NEF1-J61	0.62	RGL1	0.63	TMEM173	0.61	SAP	0.6	FSTL1	0.6				
ATP10A	0.73	CTC3	0.62	FTD	0.61	LATS2	0.64	NREP	0.75	ROXA	0.63	TMEM43	0.67	FBN1	0.63	GFPT2	0.63				
ATP8B2	0.61	CRYAB	0.66	FUT11	0.71	LAYN	0.70	NRK	0.62	RHOQ	0.62	TMEM45A	0.62	FND1C1	0.71	GLI2	0.71				
ATXN1	0.63	CSGALNACT2	0.75	FXD6	0.65	LBH	0.65	NRP2	0.70	RELPL1	0.64	TMEM47	0.65	FSTL1	0.61	GLTB2	0.61				
BACE1	0.61	CTHRC1	0.73	FZD1	0.73	LGALS1	0.72	NRXN3	0.72	RNMLB	0.62	TNRCC2	0.60	IGFBP7	0.6	IGFBP7	0.6				
BGN	0.83	CTSK	0.79	GAL3ST4	0.64	LHFPL6	0.71	NTM	0.61	RNF144A	0.73	TNS1	0.71	GGT5	0.64	ISLR	0.64				
BHLHE22	0.65	CYBB01	0.60	GAS1	0.73	LINC01279	0.73	NUAK1	0.72	RNF146	0.60	TPM1	0.67	GLI1	0.67	ITGB1	0.67				
BK1C1	0.82	CYP11B1	0.73	GAS7	0.74	LXN1	0.71	OLFM1	0.62	RNF150	0.66	TPM2	0.70	GLI2	0.64	KAA1755	0.64				
BNP3	0.68	CYP21L1	0.63	GFPT2	0.71	LOC101929122	0.65	OLFM2B	0.74	RNF117	0.75	TRO	0.66	GLTB2	0.63	LOX1	0.63				
BN2	0.75	DNAI2	0.67	GJA1	0.62	LOX	0.63	OMD	0.67	ROBO1	0.67	TRPC1	0.71	HIC1	0.63	LRRK32	0.63				
BNP2	0.63	DAC11	0.75	GLI2	0.63	LOX1	0.66	PALD	0.74	ROR2	0.69	TRPS1	0.66	HTRA3	0.63	LTPB2	0.63				
BOC	0.76	DCLK1	0.64	GLI3	0.72	LROH2	0.61	PARO8B	0.62	RSPO3	0.68	TSO2	0.72	IGFBP7	0.63	LUM	0.63				
C14orf132	0.61	DCLK2	0.61	GLPR1	0.63	LRP12	0.61	PARVA	0.70	RTN1	0.62	TSO3	0.74	IGFBP7-AS1	0.62	MMP2	0.62				
C1orf126	0.60	DCN	0.74	GLI2	0.77	LRRK32	0.69	PRX3	0.63	RUFY3	0.62	TSPAN4	0.64	ISLR	0.77	MOXD1	0.77				
C1orf54	0.63	DDR2	0.75	GLTB2	0.79	LRRN4CL	0.65	PCOLCE	0.68	RUNX1T1	0.80	TSPAN9	0.65	ISLR2	0.62	MRC2	0.62				
C1QTNF3	0.63	DIENND5A	0.68	GNAL	0.63	LSAMP	0.63	PDE3A	0.64	SIPR3	0.64	TUB	0.62	ITGA11	0.62	M8R48	0.62				
C1R	0.78	DPP1	0.68	GNB4	0.74	LTPB1	0.69	PDGFC	0.67	SAMDA4	0.61	TUBA1A	0.78	ITGB1	0.76	NALCN	0.76				
C1S	0.77	DPP3	0.61	GNK5	0.63	LTPB2	0.78	PDGFRB	0.74	SCARF2	0.64	TUBB8	0.68	KAA1755	0.65	NOX4	0.65				
C3	0.62	DREK1	0.68	GNK6	0.75	LTPB3	0.75	POGFR	0.76	SCRG1	0.65	THSD7	0.63	LOX1	0.64	NREP	0.64				
C6orf3	0.65	DNAH3D5	0.70	GRM1	0.75	LUM	0.79	POLM2	0.62	SOC2	0.69	THSD1	0.60	LRRK32	0.72	OLFM2B	0.72				
C9orf88	0.60	DOCK11	0.67	GLUCY1A3	0.74	MACF1	0.63	POLM3	0.75	SELENOM	0.69	UBE2E2	0.72	LTPB2	0.71	OMD	0.71				
CALD1	0.82	DOK5	0.67	GLUCY1B3	0.74	MAF8	0.66	POLM4	0.63	SERPINE2	0.66	UBE2Q2	0.63	LUM	0.71	POGFR8	0.71				
CAUHM2	0.75	DPSL3	0.85	GXYL2	0.77	MAGO-AS3	0.71	POZRN3	0.69	SERPINF1	0.80	VAT1	0.64	LZT51	0.61	PLXDC2	0.61				
CAUHM5	0.71	DSE	0.73	HDSFL3	0.76	MAN1C1	0.65	PEAL5	0.77	SERPINF1	0.75	VCAN	0.79	MMP2	0.63	SCARF2	0.63				
CAMSAP2	0.63	DZP1	0.69	HECTD2	0.68	MAP1A	0.68	PEAK1	0.62	SETP1	0.61	VEGFC	0.61	MOXD1	0.74	SERPINF1	0.74				
CAP2	0.64	ECM2	0.75	HEG1	0.75	MAP1B	0.66	PEI3	0.62	SFRP2	0.74	VGLL3	0.70	SPARC	0.63	SPARC	0.63				
CARMN	0.63	EDNRA	0.69	HEY1	0.65	MAP3K20	0.66	PHLDA3	0.64	SGCD	0.67	VIM	0.68	M8R48	0.71	THSD2	0.71				
CAVIN1	0.79	EFEMP1	0.67	HMCN1	0.81	MAP3K3	0.67	PHLDB2	0.63	SGCE	0.68	VSTM4	0.66	NALCN	0.7	THY1	0.7				
CAVIN3	0.61	EFEMP2	0.85	HDOK3	0.65	MAP4	0.65	PKD2	0.78	SH3PKD2A	0.63	WISP1	0.69	NOX4	0.74	TIMP2	0.74				
CCDC80	0.78	EFS	0.65	HDXB2	0.62	MARVELD1	0.74	PLAGL1	0.62	SH3PKD2B	0.64	WWTR1	0.70	NREP	0.68	VCAN	0.68				
CD109	0.61	EID1	0.62	HSD11B1	0.64	MDRC	0.63	PLPFR4	0.62	SH3RF3	0.61	ZBTB20	0.60	OLFM2B	0.63	VEGFC	0.63				
CDCA7BP3	0.65	ELN	0.71	HSPB2	0.65	MEIS1	0.65	SLC3A3	0.60	SLC3A3	0.70	ZBTB4	0.61	OMD	0.61	WFS21	0.61				
CDH11	0.87	EMILN1	0.72	HTRA1	0.62	MEIOX2	0.65	PLXDC2	0.84	SLIT2	0.73	ZCCHC24	0.75	POGFR	0.66	PLXDC2	0.66				
CDK14	0.63	EMIL1	0.69	IFIT1	0.63	MFAP2	0.66	SMOCC2	0.74	ZEB1	0.74	ZEB1	0.74	PLXDC2	0.67	PLXDC2	0.67				
CD01	0.61	ENDX1	0.61	IGFBP4	0.66	MFAP5	0.66	PODN	0.67	SOCS5	0.74	ZHK3	0.64	SCARF2	0.66	SCARF2	0.66				
CDON	0.61	EPDR1	0.63	IGFBP6	0.67	MGP	0.77	PRDM2	0.63	SPARC	0.83	ZFPK2	0.74	SERPINF1	0.65	SERPINF1	0.65				
CHRD1	0.60	EPHA3	0.66	IGFBP7	0.72	MIR100HG	0.74	PRDM6	0.65	SPARCL1	0.64	ZNF35	0.68	SPARC	0.63	SPARC	0.63				
CLEC11A	0.73	EVC	0.83	IL17RD	0.62	MIR99AHG	0.67	PRELP	0.75	SPART	0.65	ZNF423	0.73	THSD2	0.7	THSD2	0.7				
CLIC4	0.74	EVC2	0.61	IL1K1	0.60	MMP2	0.74	PRICKLE1	0.69	SPN1	0.65	ZNF436	0.73	THY1	0.6	THY1	0.6				
CLIP3	0.62	FAM198A	0.62	INHBA	0.73	MN1	0.76	PRICKLE2	0.61	SPOCK1	0.85	ZNF521	0.66	TIMP2	0.65	TIMP2	0.65				
CLP4	0.63	FAM198B	0.75	INHAT	0.66	MORF4L1	0.65	PRKD1	0.76	SPON1	0.66	ZNF532	0.63	VCAN	0.61	VCAN	0.61				
CLMP	0.66	FAM19A5	0.69	IRF2BP1	0.67	MOXD1	0.82	PRNG1	0.64	SPN	0.74	ZNF667-AS1	0.61	VEGFC	0.6	VEGFC	0.6				
CMT3	0.75	FAM208A	0.66	ISLR	0.87	MPDZ	0.74	PRNP	0.61	STGALNACS	0.63	ZNF521	0.6	ZNF521	0.6	ZNF521	0.6				

A**B**

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR	found	ratio
Extracellular matrix organisation	14/329	0.023	1.07E-09	3.63e-07	77/318	0.025
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	9/127	0.009	1.85e-08	3.14e-06	9/14	0.001
Elastic fibre formation	6/46	0.003	1.49e-06	1.68e-05	12/17	0.001
Post-translational protei phosphorylation	7/109	0.008	1.48e-06	1.11e-04	1/1	8.01e-05
Molecules associated with elastic fibres	5/38	0.003	1.64e-06	1.11e-04	5/10	8.01e-04
ECM proteoglycans	5/79	0.005	5.42e-05	0.003	5/23	0.002
Diseases associated with glycosaminoglycan metabolism	4/55	0.004	1.86e-04	0.009	12/29	0.002
Degradation of extracellular matrix	5/148	0.01	9.55e-04	0.026	34/105	0.008

Figure S4. Genes and pathways correlated with SFRP4. (A) Genes commonly expressed in the Australian and TCGA cohorts were identified (Pearson correlation >0.6). (B) Top 8 functional pathways found to be enriched after further analysis of these genes using Reactome.

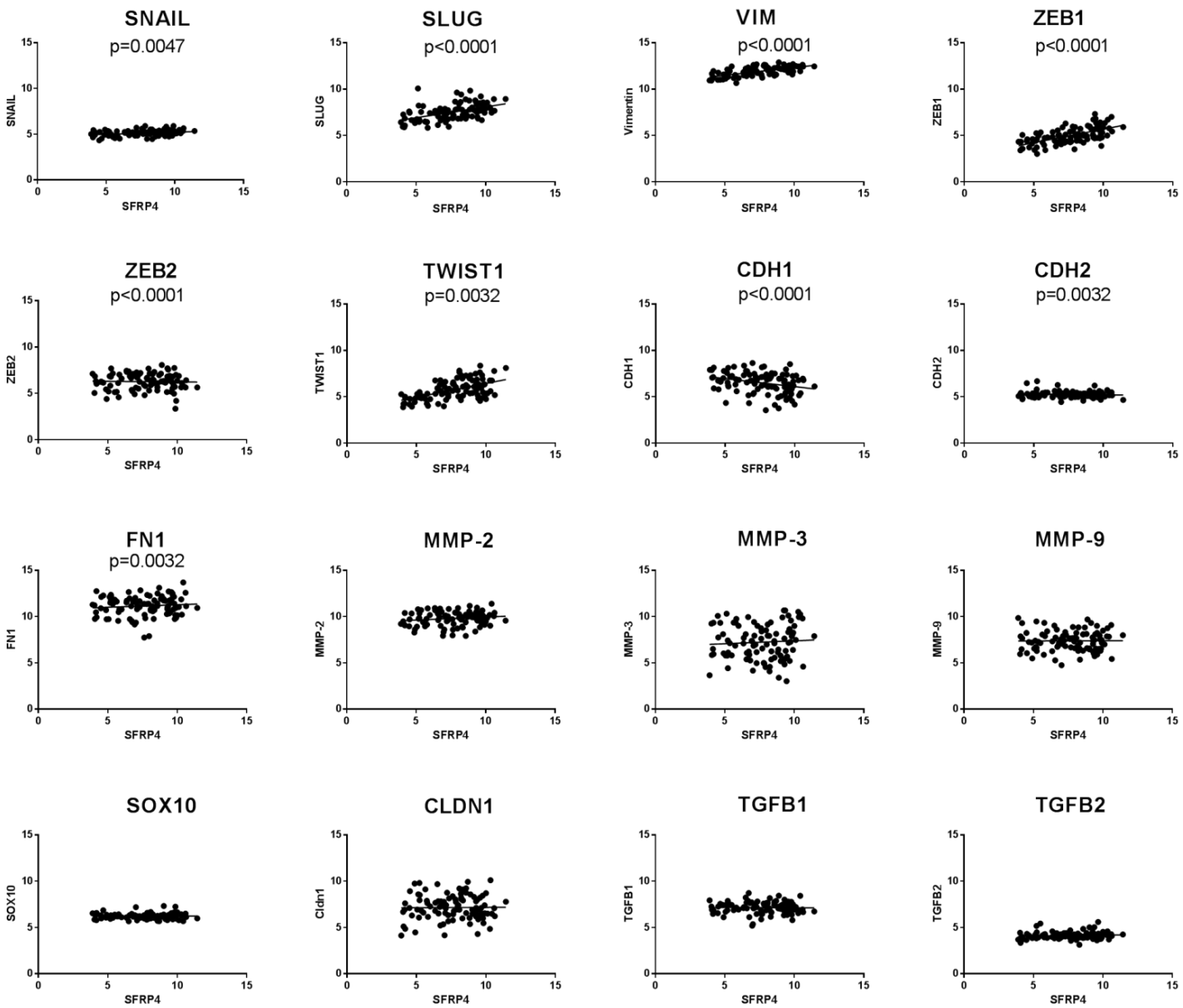


Figure S5. Correlation of SFRP4 expression with EMT related genes. Affymetrix U133 plus 2 mRNA data from the Australian dataset was used to correlate expression of SFRP4 with key EMT related genes

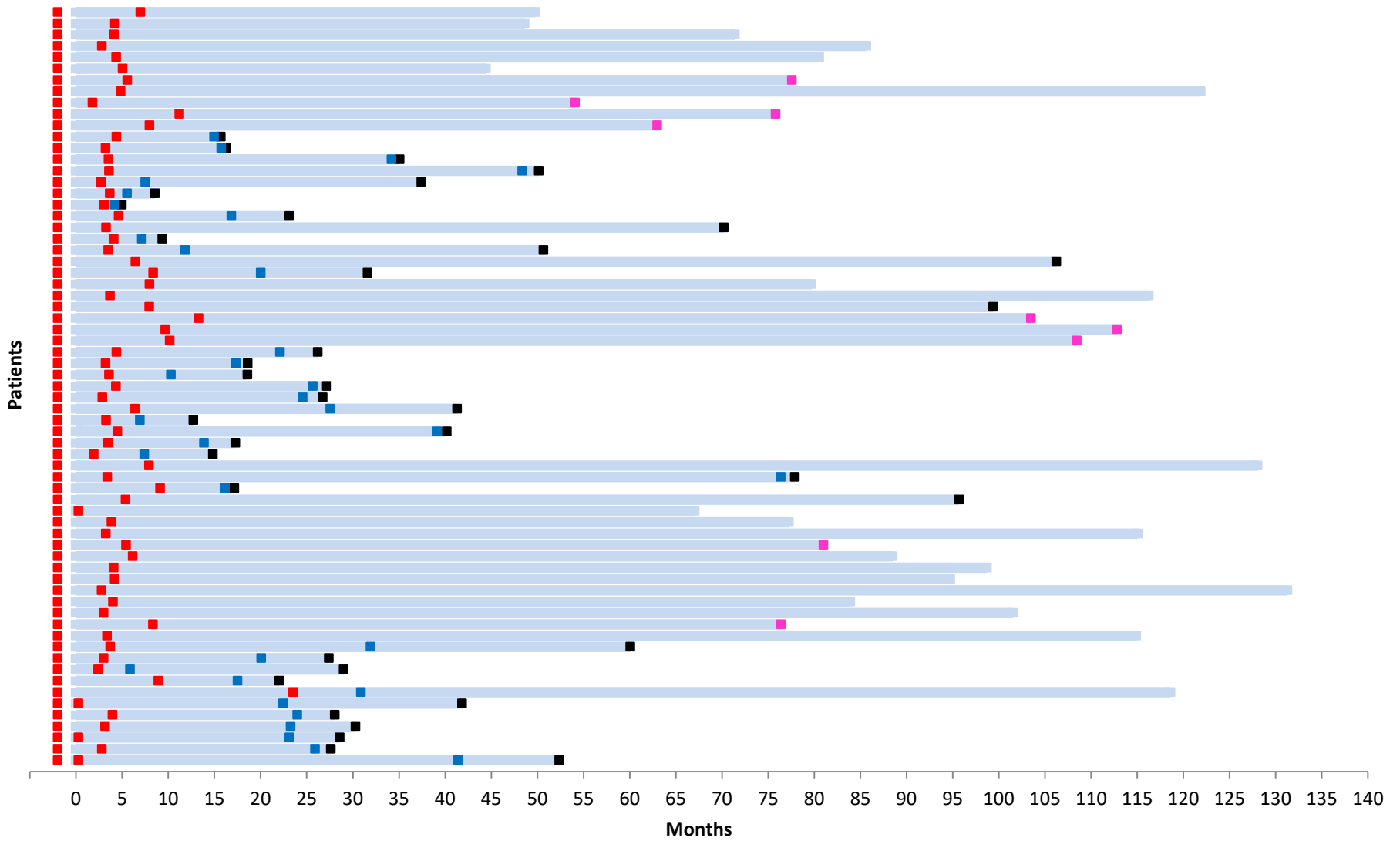


Figure S6. Swimmer plots outlining timelines for sample collection, recurrence and patient prognosis in the Australian cohort (n= 67). Zero months indicates the date of surgery. Key: Red= plasma sample collections; blue= time of clinically documented recurrence; black = overall survival; pink =lost to follow up