SUPPLEMENTARY DATA FOR:

P14ARF inhibits human glioblastoma-induced angiogenesis by regulating the expression of TIMP3

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S Table 1: List of angiogenesis –related genes modulated by P14ARF in A5 cells.

A5 human glioma cells were grown in duplicate plates to 80% confluence; then, the *P14ARF* gene was induced in one plate by exposing the cells to doxycycline (2ug/ml) for 48hrs after which the cells were lysed and total RNA prepared from two independent experiments using Trizol (Invitrogen, Carlsbad, CA). The integrity of the RNA was first monitored by agarose gel electrophoresis and then reverse transcribed to DNA, followed by *in vitro* transcription using BioArray High Yield RNA transcript labelling kit (Enzo, Farmingdale, NY). Biotin labelled cRNA was then fragmented according to the Affymetrix standard protocols. cRNA probes were hybridized overnight at 45ºC to the Human Genome U133A arrays (Affymetrix, Santa Clara, CA), washed in a Fluidics Workstation, and scanned according to the manufacturer's protocols. The Affymetrix CEL files were normalized using the robust multi-chip analysis (RMA) method. After data normalization a list of genes up- and down-regulated upon *P14ARF* induction (dox) was generated by comparing gene expression profiles of A5 (+dox vs ctrl) using Gen Traffic software. **Microarray gene expression data were deposited at EMBL-EBI Array Express under accession E-MEXP-3476.** Angiogenesis-related genes with hybridization detection signal above 100 (background) and significantly modulated by P14ARF in A5 cells (dox vs ctrl) are highlighted. Changes in gene expression levels are considered significant when p-value < 0.05.

Probe set ID	Gene Symbol	A5 Ctrl	A5+dox	Fold induction	p-value	Entrez Gene Name
208042_at	**AGGF1**	**99.75**	**74.45**	**0.75**	***0.289***	**angiogenic factor with G patch and FHA domains 1**
218534_s_at	**AGGF1**	**185.6**	**171.7**	**0.93**	***0.524***	angiogenic factor with G patch and FHA domains 1
210710_at	**AGGF1**	**41.9**	**51.8**	**1.24**	***0.577***	angiogenic factor with G patch and FHA domains 1
205609_at	**ANGPT1**	**27.2**	**21.95**	**0.81**	***0.469***	angiopoietin 1
205609_at	**ANGPT1**	**27.2**	**21.95**	**0.81**	***0.469***	angiopoietin 1
205608_s_at	**ANGPT1**	**112.7**	**98.55**	**0.87**	***0.705***	angiopoietin 1
205608_s_at	**ANGPT1**	**112.7**	**98.55**	**0.87**	***0.705***	angiopoietin 1
221009_s_at	**ANGPTL4**	**26.45**	**76.55**	**2.89**	***0.027***	angiopoietin-like 4
205290_s_at	**BMP2**	**57.5**	**105.2**	**1.83**	***0.014***	bone morphogenetic protein 2
205289_at	**BMP2**	**51.9**	**79**	**1.52**	***0.290***	bone morphogenetic protein 2

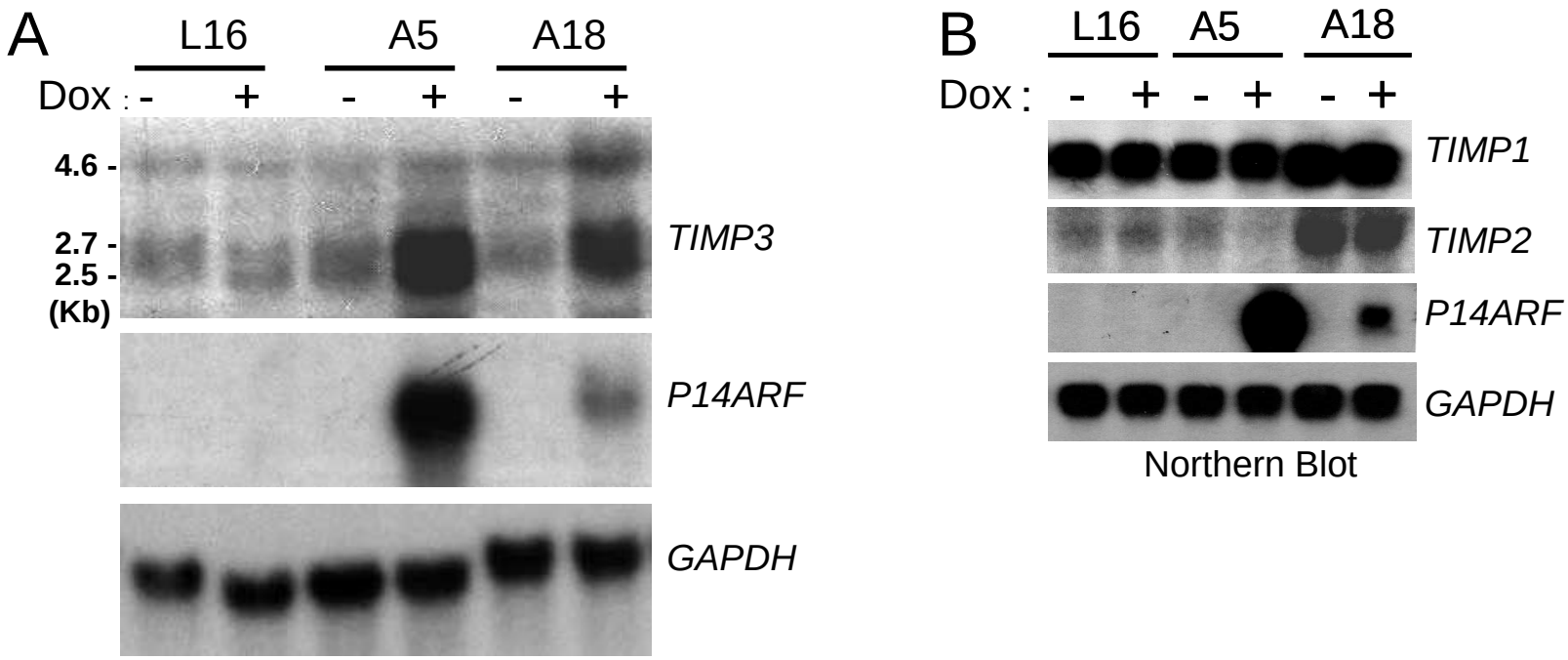
200920_s_at	**BTG1**	**733.2**	**1335.35**	**1.82**	***0.064***	B-cell translocation gene 1, anti-proliferative
200921_s_at	**BTG1**	**395.7**	**624.4**	**1.58**	***0.372***	B-cell translocation gene 1, anti-proliferative
216598_s_at	**CCL2**	**2715**	**2118.85**	**0.78**	***0.777***	chemokine (C-C motif) ligand 2
201926_s_at	**CD55**	**297.8**	**543.1**	**1.82**	***0.010***	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
201925_s_at	**CD55**	**125.15**	**168.95**	**1.35**	***0.384***	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
200984_s_at	**CD59**	**2249.3**	**2918.6**	**1.30**	***0.029***	CD59 molecule, complement regulatory protein
212463_at	**CD59**	**436.8**	**497.75**	**1.14**	***0.006***	CD59 molecule, complement regulatory protein
200983_x_at	**CD59**	**3847**	**5416.85**	**1.41**	***0.055***	CD59 molecule, complement regulatory protein
200985_s_at	**CD59**	**1777.6**	**2266.1**	**1.27**	***0.213***	CD59 molecule, complement regulatory protein
209082_s_at	**COL18A1**	**146.85**	**82.95**	**0.56**	***0.117***	collagen, type XVIII, alpha 1
209081_s_at	**COL18A1**	**135.4**	**70.95**	**0.52**	***0.297***	collagen, type XVIII, alpha 1
216898_s_at	**COL4A3**	**17.4**	**13**	**0.75**	***0.131***	collagen, type IV, alpha 3 (Goodpasture antigen)
216896_at	**COL4A3**	**2.85**	**11.85**	**4.16**	***0.424***	collagen, type IV, alpha 3 (Goodpasture antigen)
216368_s_at	**COL4A3**	**46.95**	**32**	**0.68**	***0.479***	collagen, type IV, alpha 3 (Goodpasture antigen)
214641_at	**COL4A3**	**17.1**	**10.65**	**0.62**	***0.583***	collagen, type IV, alpha 3 (Goodpasture antigen)
216367_at	**COL4A3**	**23.2**	**12.65**	**0.55**	***0.591***	collagen, type IV, alpha 3 (Goodpasture antigen)
216893_s_at	**COL4A3**	**11.05**	**12.5**	**1.13**	***0.879***	collagen, type IV, alpha 3 (Goodpasture antigen)
222073_at	**COL4A3**	**16.15**	**16.5**	**1.02**	***0.973***	collagen, type IV, alpha 3 (Goodpasture antigen)
204470_at	**CXCL1**	**354.7**	**860.1**	**2.42**	***0.524***	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
204533_at	**CXCL10**	**71.5**	**67.95**	**0.95**	***0.447***	chemokine (C-X-C motif) ligand 10
209687_at	**CXCL12**	**6.75**	**16.5**	**2.44**	***0.128***	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
203666_at	**CXCL12**	**78**	**54.45**	**0.70**	***0.137***	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
207379_at	**EDIL3**	**155.6**	**102.75**	**0.66**	***0.266***	EGF-like repeats and discoidin I-like domains 3
202669_s_at	**EFNB2**	**51.85**	**121.75**	**2.35**	***0.302***	ephrin-B2
202668_at	**EFNB2**	**100.15**	**107.45**	**1.07**	***0.863***	ephrin-B2
206254_at	**EGF**	**23.4**	**20.5**	**0.88**	***0.530***	epidermal growth factor (beta-urogastrone)
216680_s_at	**EPHB4**	**87.2**	**66.65**	**0.76**	***0.616***	EPH receptor B4
202894_at	**EPHB4**	**86.3**	**79.45**	**0.92**	***0.865***	EPH receptor B4
205767_at	**EREG**	**7.75**	**39.35**	**5.08**	***0.224***	epiregulin
205110_s_at	**FGF13**	**166.25**	**147.25**	**0.89**	***0.560***	fibroblast growth factor 13
204421_s_at	**FGF2**	**68.2**	**97**	**1.42**	***0.145***	fibroblast growth factor 2 (basic)

204422_s_at	**FGF2**	**43**	**23.55**	**0.55**	***0.360***	fibroblast growth factor 2 (basic)
211719_x_at	**FN1**	**2198.8**	**1891**	**0.86**	***0.569***	fibronectin 1
216442_x_at	**FN1**	**2569.05**	**2236.85**	**0.87**	***0.640***	fibronectin 1
214701_s_at	**FN1**	**265.7**	**249.95**	**0.94**	***0.662***	fibronectin 1
210495_x_at	**FN1**	**2758.95**	**2581.55**	**0.94**	***0.683***	fibronectin 1
212464_s_at	**FN1**	**2091.4**	**2050.9**	**0.98**	***0.840***	fibronectin 1
214702_at	**FN1**	**22.85**	**20.55**	**0.90**	***0.881***	fibronectin 1
204948_s_at	**FST**	**530.7**	**1008.3**	**1.90**	***0.444***	follistatin
207345_at	**FST**	**76.25**	**100.8**	**1.32**	***0.494***	follistatin
200678_x_at	**GRN**	**680.15**	**985.35**	**1.45**	***0.013***	granulin
211284_s_at	**GRN**	**231.8**	**376**	**1.62**	***0.093***	granulin
216041_x_at	**GRN**	**637.6**	**857.85**	**1.35**	***0.210***	granulin
210755_at	**HGF**	**15.5**	**9.45**	**0.61**	***0.354***	hepatocyte growth factor (hepapoietin A; scatter factor)
209960_at	**HGF**	**1.25**	**6.65**	**5.32**	***0.441***	hepatocyte growth factor (hepapoietin A; scatter factor)
210998_s_at	**HGF**	**2.3**	**3**	**1.30**	***0.645***	hepatocyte growth factor (hepapoietin A; scatter factor)
210997_at	**HGF**	**2.1**	**2**	**0.95**	***0.698***	hepatocyte growth factor (hepapoietin A; scatter factor)
209961_s_at	**HGF**	**84.3**	**85**	**1.01**	***0.980***	hepatocyte growth factor (hepapoietin A; scatter factor)
200989_at	**HIF1A**	**1893.75**	**2348.3**	**1.24**	***0.608***	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
219403_s_at	**HPSE**	**120.55**	**179.55**	**1.49**	***0.369***	heparanase
208937_s_at	**ID1**	**320.9**	**297.35**	**0.93**	***0.869***	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
207160_at	**IL12A**	**16.2**	**21.05**	**1.30**	***0.201***	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
207901_at	**IL12B**	**11.4**	**13.75**	**1.21**	***0.561***	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
39402_at	**IL1B**	**61**	**375.8**	**6.16**	***0.332***	interleukin 1, beta
205067_at	**IL1B**	**80.45**	**593.45**	**7.38**	***0.352***	interleukin 1, beta
205207_at	**IL6**	**42.05**	**48.05**	**1.14**	***0.778***	interleukin 6 (interferon, beta 2)
211506_s_at	**IL8**	**46.15**	**446.45**	**9.67**	***0.394***	interleukin 8
202859_x_at	**IL8**	**114.15**	**639.65**	**5.60**	***0.423***	interleukin 8
202351_at	**ITGAV**	**475.9**	**324.1**	**0.68**	***0.239***	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
204628_s_at	**ITGB3**	**169.5**	**178.35**	**1.05**	***0.034***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)

215240_at	**ITGB3**	**54.95**	**46**	**0.84**	***0.421***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
204627_s_at	**ITGB3**	**338.45**	**284.3**	**0.84**	***0.480***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
204625_s_at	**ITGB3**	**192.55**	**211.15**	**1.10**	***0.792***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
211579_at	**ITGB3**	**17.45**	**19.95**	**1.14**	***0.819***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
216261_at	**ITGB3**	**73.45**	**80.15**	**1.09**	***0.855***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
204626_s_at	**ITGB3**	**136.35**	**134.1**	**0.98**	***0.927***	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
209098_s_at	**JAG1**	**34.3**	**66.95**	**1.95**	***0.039***	jagged 1 (Alagille syndrome)
216268_s_at	**JAG1**	**75.95**	**175.75**	**2.31**	***0.072***	jagged 1 (Alagille syndrome)
209099_x_at	**JAG1**	**67.35**	**210**	**3.12**	***0.098***	jagged 1 (Alagille syndrome)
209097_s_at	**JAG1**	**3.35**	**6.25**	**1.87**	***0.467***	jagged 1 (Alagille syndrome)
211124_s_at	**KITLG**	**10.55**	**34.7**	**3.29**	***0.098***	KIT ligand
207029_at	**KITLG**	**29.25**	**54.25**	**1.85**	***0.326***	KIT ligand
209035_at	**MDK**	**179.1**	**183.95**	**1.03**	***0.873***	midkine (neurite growth-promoting factor 2)
201069_at	**MMP2**	**4083.8**	**3300.8**	**0.81**	***0.056***	matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
203936_s_at	**MMP9**	**120.25**	**101.35**	**0.84**	***0.508***	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
205247_at	**NOTCH4**	**46.7**	**38.35**	**0.82**	***0.712***	Notch homolog 4 (Drosophila)
212298_at	**NRP1**	**95.35**	**166.9**	**1.75**	***0.035***	neuropilin 1
210615_at	**NRP1**	**20.45**	**13.25**	**0.65**	***0.643***	neuropilin 1
210841_s_at	**NRP2**	**92.75**	**131.25**	**1.42**	***0.025***	neuropilin 2
214632_at	**NRP2**	**60.05**	**126.6**	**2.11**	***0.217***	neuropilin 2
210842_at	**NRP2**	**6.65**	**13**	**1.95**	***0.356***	neuropilin 2
211844_s_at	**NRP2**	**3.8**	**24.55**	**6.46**	***0.425***	neuropilin 2
219367_s_at	**NRP2**	**87.95**	**90**	**1.02**	***0.928***	neuropilin 2
205463_s_at	**PDGFA**	**639.95**	**499.2**	**0.78**	***0.601***	platelet-derived growth factor alpha polypeptide
216867_s_at	**PDGFA**	**147.7**	**181.3**	**1.23**	***0.798***	platelet-derived growth factor alpha polypeptide
219304_s_at	**PDGFD**	**66.7**	**47**	**0.70**	***0.137***	platelet derived growth factor D
209652_s_at	**PGF**	**20.5**	**14.7**	**0.72**	***0.415***	placental growth factor
215179_x_at	**PGF**	**378.95**	**323.25**	**0.85**	***0.487***	placental growth factor
205479_s_at	**PLAU**	**39.8**	**60.7**	**1.53**	***0.252***	plasminogen activator, urokinase
211668_s_at	**PLAU**	**54.1**	**42.55**	**0.79**	***0.357***	plasminogen activator, urokinase
209977_at	**PLG**	**8.05**	**3.8**	**0.47**	***0.333***	plasminogen
219700_at	**PLXDC1**	**26.45**	**31.8**	**1.20**	***0.603***	plexin domain containing 1

214081_at	**PLXDC1**	**30.25**	**28.65**	**0.95**	***0.882***	plexin domain containing 1
205445_at	**PRL**	**50.05**	**35.05**	**0.70**	***0.322***	prolactin
205127_at	**PTGS1**	**8.7**	**11.75**	**1.35**	***0.051***	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
215813_s_at	**PTGS1**	**101.95**	**66.75**	**0.65**	***0.004***	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
205128_x_at	**PTGS1**	**64.65**	**84.2**	**1.30**	***0.213***	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
212099_at	**RHOB**	**327.9**	**418.45**	**1.28**	***0.461***	ras homolog gene family, member B
206050_s_at	**RNH1**	**754.45**	**782.35**	**1.04**	***0.261***	ribonuclease/angiogenin inhibitor 1
216798_at	**RNH1**	**40.85**	**32.4**	**0.79**	***0.285***	ribonuclease/angiogenin inhibitor 1
209359_x_at	**RUNX1**	**22.8**	**103.35**	**4.53**	***0.120***	runt-related transcription factor 1
211180_x_at	**RUNX1**	**36.2**	**104.9**	**2.90**	***0.189***	runt-related transcription factor 1
210805_x_at	**RUNX1**	**3.4**	**5.35**	**1.57**	***0.294***	runt-related transcription factor 1
211182_x_at	**RUNX1**	**9.8**	**25.85**	**2.64**	***0.325***	runt-related transcription factor 1
211620_x_at	**RUNX1**	**9.7**	**30.2**	**3.11**	***0.331***	runt-related transcription factor 1
209360_s_at	**RUNX1**	**141.85**	**165.7**	**1.17**	***0.474***	runt-related transcription factor 1
208129_x_at	**RUNX1**	**36.95**	**41.35**	**1.12**	***0.550***	runt-related transcription factor 1
210365_at	**RUNX1**	**43.1**	**57.1**	**1.32**	***0.594***	runt-related transcription factor 1
217263_x_at	**RUNX1**	**26.1**	**20.3**	**0.78**	***0.719***	runt-related transcription factor 1
211181_x_at	**RUNX1**	**39.6**	**52.2**	**1.32**	***0.770***	runt-related transcription factor 1
211179_at	**RUNX1**	**2.15**	**2.3**	**1.07**	***0.827***	runt-related transcription factor 1
219257_s_at	**SPHK1**	**227**	**252.95**	**1.11**	***0.860***	sphingosine kinase 1
205015_s_at	**TGFA**	**152.2**	**263.7**	**1.73**	***0.041***	transforming growth factor, alpha
205016_at	**TGFA**	**711.65**	**1027.9**	**1.44**	***0.101***	transforming growth factor, alpha
211258_s_at	**TGFA**	**168.2**	**261.85**	**1.56**	***0.136***	transforming growth factor, alpha
203085_s_at	**TGFB1**	**253.3**	**442.25**	**1.75**	***0.027***	transforming growth factor, beta 1
203084_at	**TGFB1**	**6.9**	**5.7**	**0.83**	***0.633***	transforming growth factor, beta 1
201109_s_at	**THBS1**	**197**	**473.4**	**2.40**	***0.153***	thrombospondin 1
201108_s_at	**THBS1**	**305.85**	**528.5**	**1.73**	***0.218***	thrombospondin 1
201110_s_at	**THBS1**	**80**	**135.2**	**1.69**	***0.223***	thrombospondin 1
215775_at	**THBS1**	**76**	**81.25**	**1.07**	***0.690***	thrombospondin 1
203083_at	**THBS2**	**622.45**	**678.85**	**1.09**	***0.536***	thrombospondin 2
201666_at	**TIMP1**	**2268.7**	**3163.8**	**1.39**	***0.063***	TIMP metallopeptidase inhibitor 1

203167_at	**TIMP2**	**495.45**	**391.95**	**0.79**	***0.467***	TIMP metallopeptidase inhibitor 2
201149_s_at	**TIMP3**	**674.9**	**2403.15**	**3.56**	***0.021***	TIMP metallopeptidase inhibitor 3
201147_s_at	**TIMP3**	**474.5**	**1532.25**	**3.23**	***0.005***	TIMP metallopeptidase inhibitor 3
201148_s_at	**TIMP3**	**433.1**	**1160**	**2.68**	***0.027***	TIMP metallopeptidase inhibitor 3
204858_s_at	**TYMP**	**100**	**134.55**	**1.35**	***0.142***	thymidine phosphorylase
217497_at	**TYMP**	**17.2**	**7.8**	**0.45**	***0.218***	thymidine phosphorylase
211527_x_at	**VEGFA**	**23.9**	**69.85**	**2.92**	***0.134***	vascular endothelial growth factor A
210513_s_at	**VEGFA**	**124.85**	**154.2**	**1.24**	***0.154***	vascular endothelial growth factor A
210512_s_at	**VEGFA**	**42.45**	**75.2**	**1.77**	***0.387***	vascular endothelial growth factor A
212171_x_at	**VEGFA**	**151.75**	**171.95**	**1.13**	***0.391***	vascular endothelial growth factor A
209946_at	**VEGFC**	**72.4**	**57.65**	**0.80**	***0.061***	vascular endothelial growth factor C

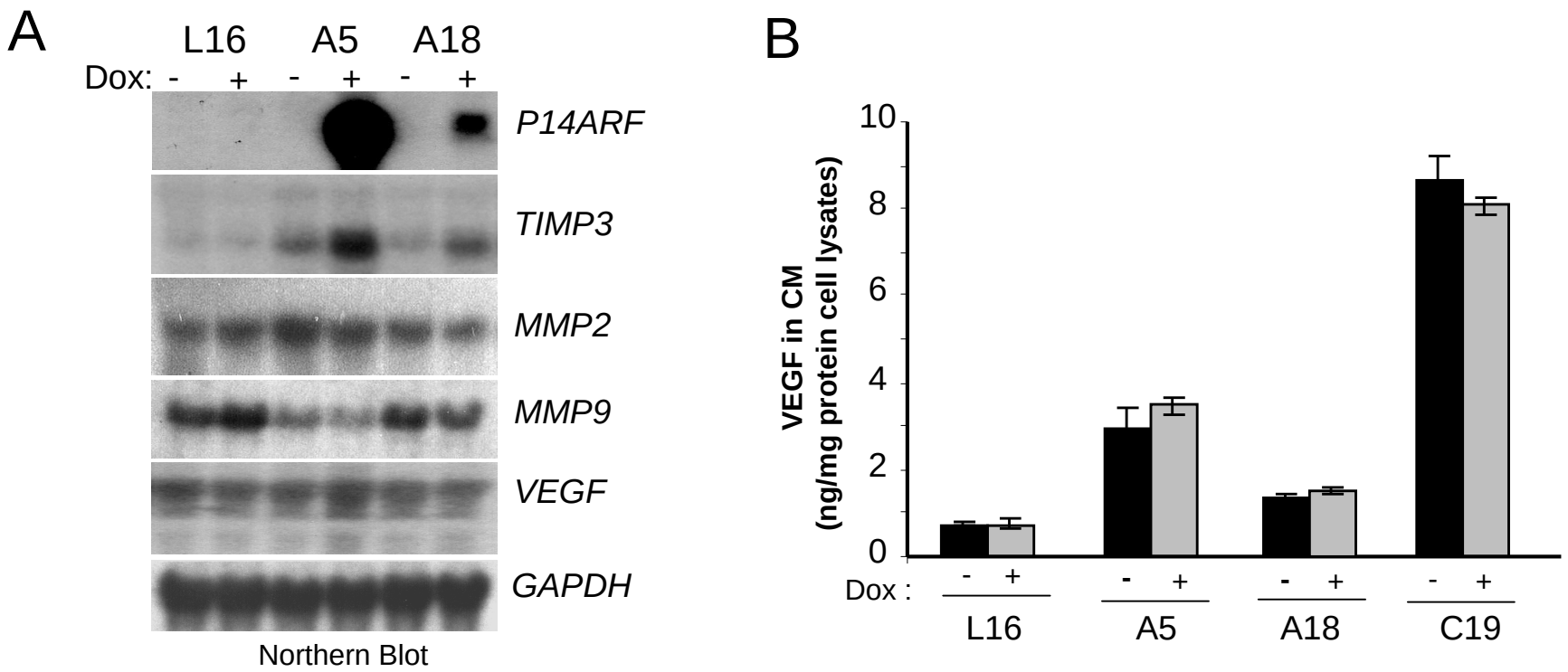


Supplementary Figure 1:

Regulation of *TIMP1, TIMP2* and *TIMP3* mRNA expression by P14ARF.

A. Independent repeat of the experiment in Figure 2A. Northern Blot showing the induction of *TIMP3* mRNA expression with doxycycline (2μg/ml for 48hrs) in *P14ARF*-expressing A5 and A18 cells. Note that dox treatment of LN229-L16 parental cells does not affect the basal expression level of all three *TIMP3* mRNA isoforms (estimated sizes in Kb are indicated).

B. Effect of P14ARF on the mRNA expression of *TIMP1* and *TIMP2* genes in A5 and A18 cells by Northern blot. Please note that neither *TIMP1* nor *TIMP2* mRNA levels were affected by *ARF* induction with dox (2μg/ml for 48hrs). *TIMP4* (not shown) was not detectable in these cells.

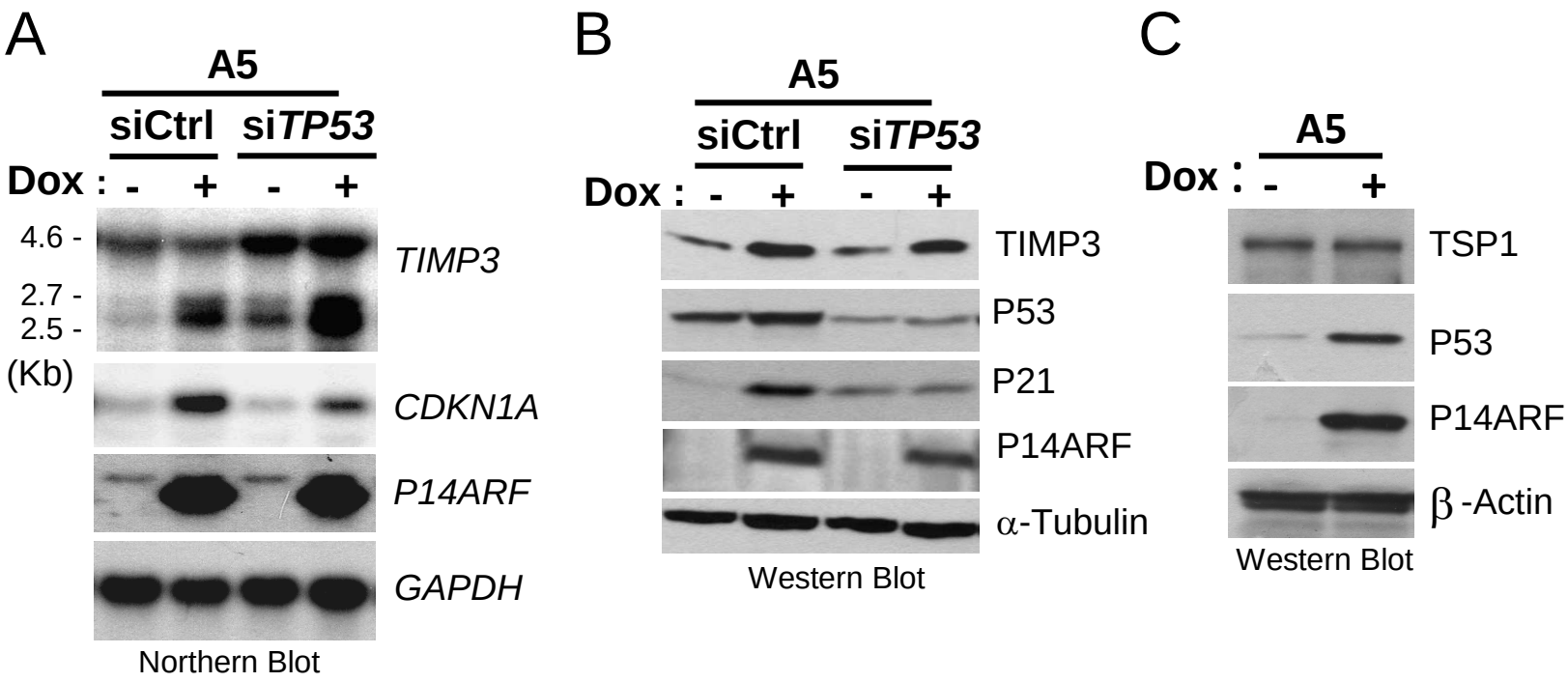


Supplementary Figure 2:

Regulation of *TIMP3, MMP2, MMP9* and *VEGF* by P14ARF.

A- Northern blot showing the effect of P14ARF expression on the transcription of *TIMP3, MMP2, MMP9* and *VEGF* genes. L16, A5 and A18 cells were treated with 2μg/ml dox for 48hrs. Note that the induction of *P14ARF* in A5 and A18 cells increases *TIMP3* mRNA levels while it does not affect the steady state of *MMP2*, *MMP9* and *VEGF* mRNAs. L16 are parental cells which do not express the *P14ARF* gene.

B- Effect of *P14ARF* expression on the levels of secreted VEGF by L16, A5, A18 and C19 cells in the culture media. Cells were plated in 12 well plate, cultured in 1 ml of serum free medium in the absence or presence of dox (2μg/ml) during 48hrs. CM was collected and VEGF was quantified using the Quantikine ELISA kit (R&D Systems, MN). No significant changes (using Student's t-test) in the levels of VEGF in culture media following the induction of *P14ARF* by dox versus controls (-dox).



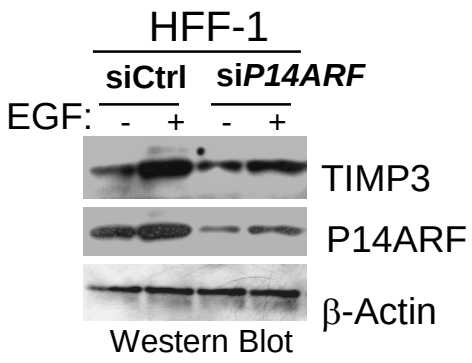
Supplementary SFigure 3:

Role of P53 in the effect of P14ARF on TIMP3 and TSP1 expression.

A- Northern blot showing that the silencing of *TP53* has no effect on p14ARF's ability to induce *TIMP3* gene expression in LN229-L16 derived A5 cells. In fact, *TP53* silencing even increased the basal expression levels of the three major *TIMP3* mRNA isoforms expressed in these cells (size in Kb estimated after Byrne JA et al, Molecular Medicine, 1:418. 1995). *P14ARF* activation with dox (2μg/ml dox for 48hrs) *and TP53* silencing (si*TP53*) synergistically activated the expression levels of the 2.5 and 2.7 Kb *TIMP3* mRNA isoforms. The expression level of *CDKN1A/P21*, a P53 transcriptional target, was used as a control to monitor that si*TP53* indeed decreased P53 levels and consequently the transcriptional activity of P53 as previously shown in **Figure 3A**.

B- Western Blot showing that siRNA mediated silencing of *TP53* dramatically decreases P53 protein levels, yet does not inhibit the induction of *TIMP3* gene expression by P14ARF in LN229 L16-derived A5 cells (2μg/ml dox for 48hrs). *TP53* silencing even slightly increased the basal expression levels of TIMP3 protein as observed in panel A at the mRNA levels. The expression of *CDKN1A/P21* is used as a control to monitor the transcriptional activity of P53.

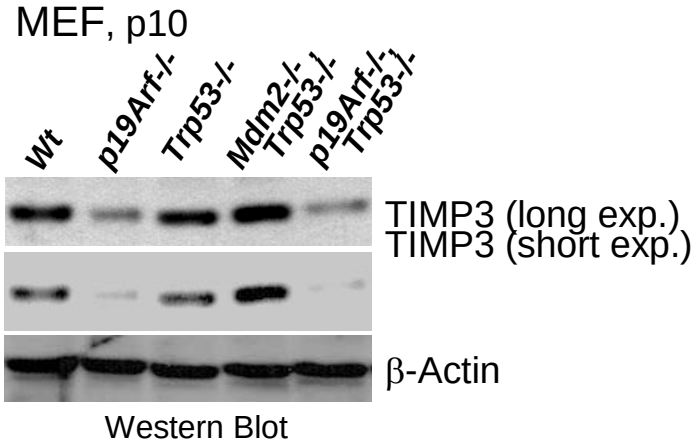
C-Western Blot showing that while P14ARF increases P53 protein levels, it failed to induce Thrombospondin (TSP1) expression in A5 cells, suggesting that the *THBS1* gene is not induced by P14ARF nor by P53 in glioma cells, confirming our previous findings with a *TP53*-inducible glioma cell line (Tenan, M. et al, J Exp Med, 191:1789-98, 2000



Supplementary Figure 4:

P14ARF silencing downregulates TIMP3 expression in HFF-1 cells

HFF-1 immortalised human fibroblasts were transfected with either siCtrl or si*P14ARF* using Lipofectamine 2000, starved for 12 hrs and then treated or not with EGF (100ng/ml) for 24 hrs. Expression of TIMP3 and P14ARF was analyzed by western blot. Note that silencing *of P14ARF* decreases EGF-induced TIMP3 levels.



Supplementary Figure 5:

Expression of *Timp3* in a different batch of MEFs (passage 10).

Expression of Timp3 in different mouse embryonic fibroblasts knocked out for genes as indicated in long and short exposures of the blot. Timp3 is decreased in *p19Arf*-null MEFs vs *Trp53*-null or *Trp53* and *Mdm2* double null *(p19Arf* positive). The absence of *p19Arf* (lane 2) or the presence of *Mdm2* (lane5) decreases TIMP3 expression.

Authors' contributions:

AZ and EGVM conceived the project and designed experiments, AZ performed and coordinated experiments in collaboration with BP for northern, western and microarray analyses (Figure 3A-C, Suppl. Table 1), MF for cornea assays (Figure 1C and 4A), DJB for pathology (Figure 1D), and AZ and EGVM interpreted the data and wrote the manuscript.