

Table W1. Quantification of Relative VEGFR2 Receptor Expression in Cytosolic and Nuclear Fractions in WM115 and WM239 Melanoma Cells Treated with Bevacizumab (50 µg/ml).

	Time (min)	pVEGFR2 Site	Exogenous VEGF	Relative Expression ± SEM	
A					
Cytosolic fractions	15	Y951	-	0.98 ± 0.24	
	30	Y951	-	0.86 ± 0.11	
	15	Y951	+	0.92 ± 0.08	
	30	Y951	+	0.94 ± 0.16	
	15	Y1175	-	1.13 ± 0.24	
	30	Y1175	-	0.87 ± 0.28	
Nuclear fractions	15	Y1175	+	1.09 ± 0.18	
	30	Y1175	+	0.88 ± 0.10	
	15	Y951	-	0.81 ± 0.07*	
	30	Y951	-	0.81 ± 0.02*	
	15	Y951	+	0.92 ± 0.22	
	30	Y951	+	1.04 ± 0.06	
Nuclear fractions	15	Y1175	-	1.00 ± 0.22	
	30	Y1175	-	0.83 ± 0.11	
	15	Y1175	+	0.80 ± 0.02*	
	30	Y1175	+	1.13 ± 0.047	
	B				
	Cytosolic fractions	15	Y951	-	0.96 ± 0.08
30		Y951	-	0.88 ± 0.16	
15		Y951	+	1.22 ± 0.18	
30		Y951	+	1.49 ± 0.60	
15		Y1175	-	0.83 ± 0.04*	
30		Y1175	-	1.00 ± 0.28	
Nuclear fractions	15	Y1175	+	1.14 ± 0.15	
	30	Y1175	+	1.29 ± 0.17	
	15	Y951	-	0.97 ± 0.13	
	30	Y951	-	1.00 ± 0.07	
	15	Y951	+	1.05 ± 0.13	
	30	Y951	+	1.27 ± 0.17	
Nuclear fractions	15	Y1175	-	0.96 ± 0.08	
	30	Y1175	-	0.89 ± 0.11	
	15	Y1175	+	1.14 ± 0.11	
	30	Y1175	+	1.15 ± 0.13	

The relative expression of native and phosphorylated VEGFR2 receptor (at Y951 and Y1175 sites) was assessed at 15- and 30-minute time points after bevacizumab addition, with or without exogenous VEGF. (A) Addition of bevacizumab had no significant ($P > .05$) effect on the phosphorylation of VEGFR2 receptor (Y951/Y1175) in cytosolic fractions 15 and 30 minutes after addition (\pm VEGF), whereas it significantly ($*P < .05$) inhibited VEGFR2 phosphorylation at Y951 (both 15 and 30 minutes after addition, -VEGF) and Y1175 (15 minutes after addition, +VEGF) in nuclear fractions in WM115 cells ($*P < .05$). (B) Similarly, addition of bevacizumab to the medium of WM239 cells only had a significant inhibitory effect on the phosphorylation of VEGFR2 at Y1175 in cytosolic fractions after 15 minutes (-VEGF; $*P < .05$). No significant change in phosphorylation of VEGFR2 (Y951/Y1175) was observed in nuclear fractions 15 and 30 minutes after bevacizumab addition (\pm VEGF; $P > .05$).