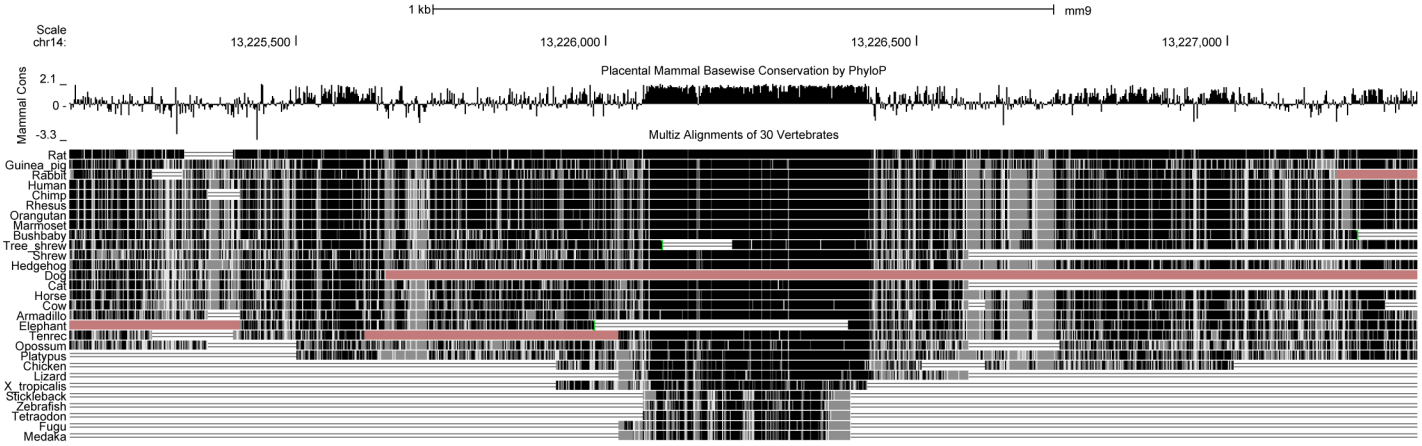Eckler Additional File 3