Online data supplement

Serum and Pulmonary Uric Acid in Pulmonary Arterial Hypertension

Running title: *Savale et al.;* Role of Urate in PAH

Laurent Savale[1,2,3], Satoshi Akagi[1,2], Ly Tu[1,2], Amélie Cumont[1,2], Raphaël Thuillet[1,2], Carole Phan[1,2], Benjamin Le Vely[1,2], Nihel Berrebeh[1,2], Alice Huertas[1,2,3], Xavier Jaïs[1,2,3], Vincent Cottin[4], Ari Chaouat[5], Cécile Tromeur[6], Athénaïs Boucly[1,2,3], Etienne Marie Jutant[1,2,3], Olaf Mercier[1,2,7], Elie Fadel[1,2,7], David Montani[1,2,3], Olivier Sitbon[1,2,3], Marc Humbert[1,2,3], Yuichi Tamura[1,2,8], Christophe Guignabert[1,2]

Figure legend

Supplemental figure S1: Expressions of xanthine oxidase (XO) and URATv1 in human pulmonary endothelial cells (ECs) in idiopathic pulmonary arterial hypertension (iPAH). (A) Representative Western blots and quantification of the XO:GADPH and URATv1:GAPDH ratios in cultured pulmonary ECs derived from control and iPAH patients. **(B)** Conditioned media from 24 h serum starved pulmonary ECs were measured for secreted UA levels using a specific uric acid assay. Data are presented as mean ± SEM (n=5-11). Comparisons were made using the nonparametric Mann-Whitney U test. * p-value < 0.05 *versus* control PA-ECs. AU = arbitrary unit.

Supplemental Figure S2: High uric acid (UA) concentrations promote reactive oxygen species (ROS) production in pulmonary artery smooth muscle cells (PA-SMCs) derived from patients with idiopathic pulmonary arterial hypertension (iPAH).
Representative images and quantification of the intracellular ROS generation using fluoregenic probes dihydroethidium (DHE) in control and iPAH PA-SMCs treated 30 minutes with UA at the indicated doses.

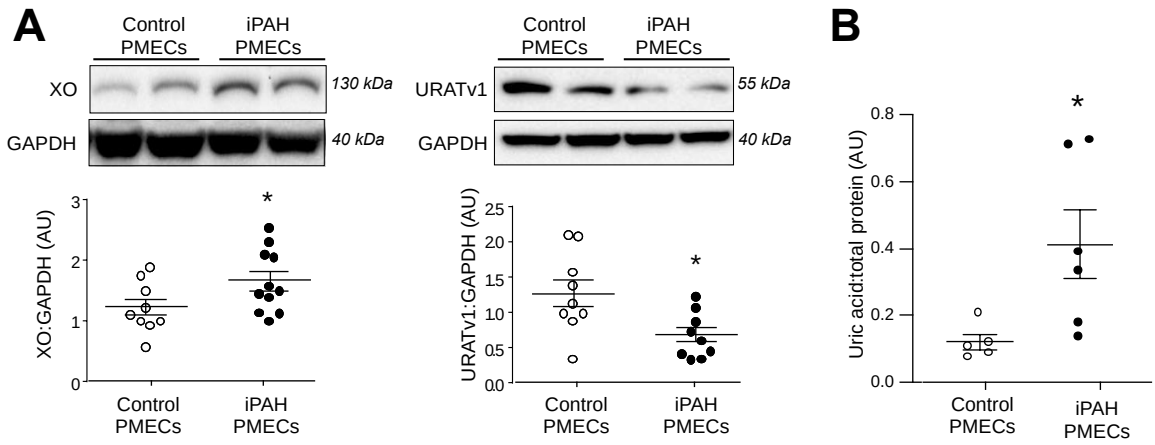
Supplemental Figure S3: (A) Values of mean pulmonary arterial pressure (mPAP), cardiac output, total pulmonary vascular resistance (PVR), and of Fulton index in control and MCT-injected rats **(B)** Representative images of α-smooth muscle (SM) actin-stained sections of distal pulmonary arteries and quantification of the percentage of wall thickness and of muscularized distal pulmonary arteries in lungs of control and MCT-injected rats. Scale bar = 20 µm in all sections. Values are means±SEM (n=4-5). Comparisons were made using 1-way ANOVA with Tukey's post hoc tests. * p-value <0.05, ** p-value <0.01, **** p-value <0.0001 compared with control rats. AU = arbitrary unit.

Supplemental Figure S4: (A) Levels of uric acid (UA) in the serum of control animals, monocrotaline (MCT)-injected at Day-21 post-MCT injection (n=7-10). **(B)** Representative Western blots and quantification of the XO:GADPH and URATv1:GAPDH ratios in lungs of control, and MCT-injected rats (n=7-10). Horizontal lines display the mean ± SEM. Comparisons were made using 1-way ANOVA with Tukey's post hoc tests/ * p-value <0.05, ** p-value <0.01, *** p-value <0.001, compared with control. AU = arbitrary unit.

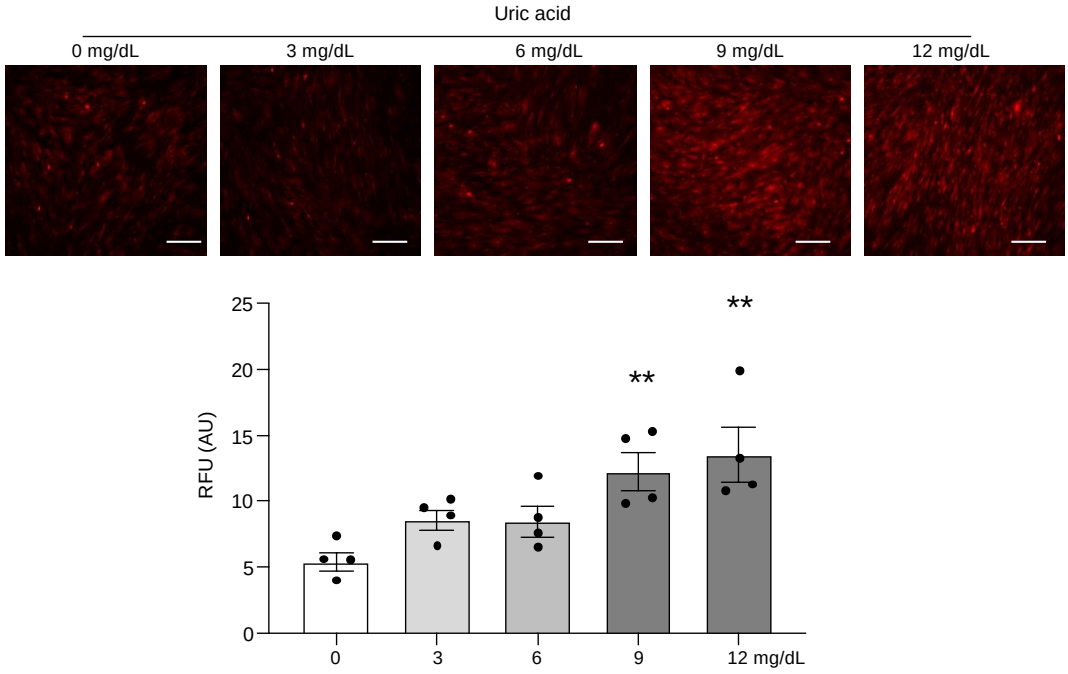
Supplemental Table S1: univariate analysis for transplant-free survival

	baseline		**First re-evaluation**	
	HR (95% CI)	**P value**	**HR (95% CI)**	**P value**
Age, *years*	1.049 (1.036-1.062)	<0.0001	1.047 (1.029-1.065)	<0.0001
Sex, *female vs male*	0.574 (0.490-0.803)	0.001	0.680 (0.404-1.145)	0.15
BMI, *kg/m^2*	1.007 (0.982-1.033)	0.58	0.976 (0.934-1.019)	0.27
Hypertension, *yes vs no*	0.535 (0.380-0.752)	0.003	3.344 (1.953-5.714)	<0.0001
Diabetis, *yes vs no*	0.749 (0.436-1.284)	0.29	2.347 (1.312-4.201)	0.004
NYHA-FC, *III-IV vs I-II*	1.625 (1.072-2.462)	0.02	3.367 (2.008-5.649)	<0.0001
6-min WD, *meters*	0.995 (0.994-0.997)	<0.0001	0.994 (0.992-0.996)	<0.0001
RAP, *mmHg*	1.025 (0.992-1.059)	0.14	1.062 (1.008-1.118)	0.02
PCWP, *mmHg*	0.991 (0.952-1.032)	0.66	1.065 (1.010-1.123)	0.02
mPAP, *mmHg*	0.987 (0.973-1.001)	0.07	1.014 (0.990-1.038)	0.27
Cardiac index, *l.min.m^2*	0.873 (0.658-1.158)	0.34	0.597 (0.405-0.880)	0.009
PVR, *WU*	0.997 (0.964-1.032)	0.88	1.073 (0.999-1.152)	0.05
BNP or NTproBNP, *high vs low*	2.088 (1.144-3.802)	0.016	5.882 (2.873-12.048)	<0.0001
Uric acid, > median value	0.846 (0.603-1.186)	0.33	1.754 (1.039-2.967)	0.03
Uric acid, mg/dL	1.001 (1.000-1.002)	0.18	1.004 (1.002-1.006)	0.0003
Diuretics, *yes vs no*			2.381 (1.262-4.504)	0.007
PAH therapies, *mono vs combo*	-	-	0.769 (0.511-1.534)	0.66

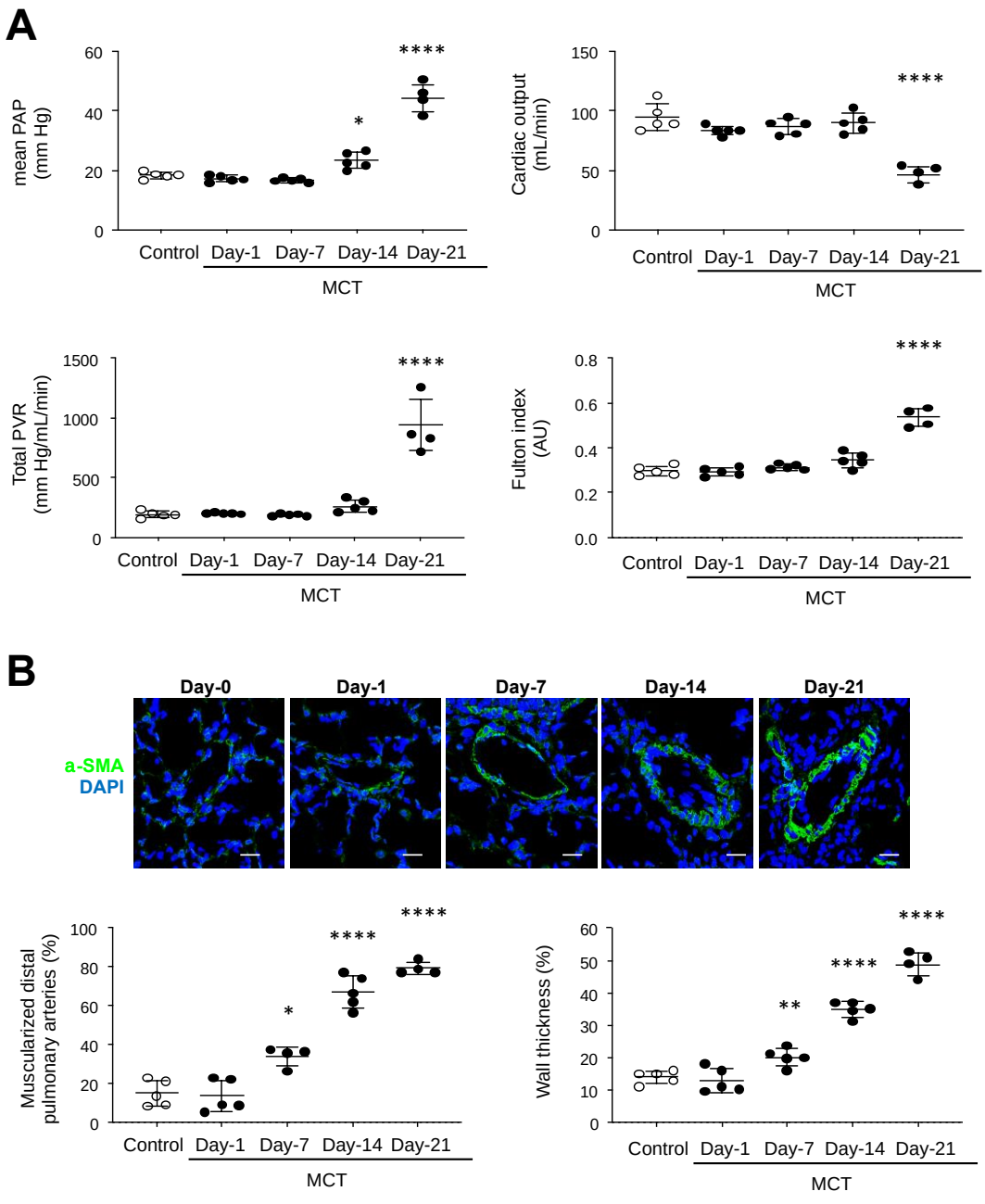
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4