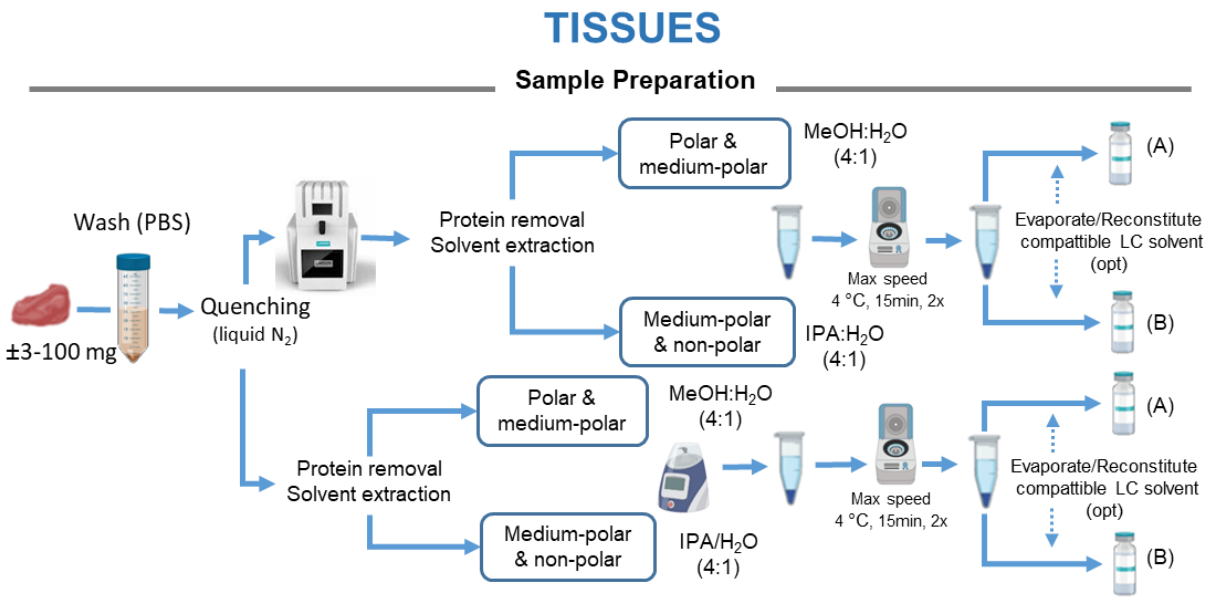
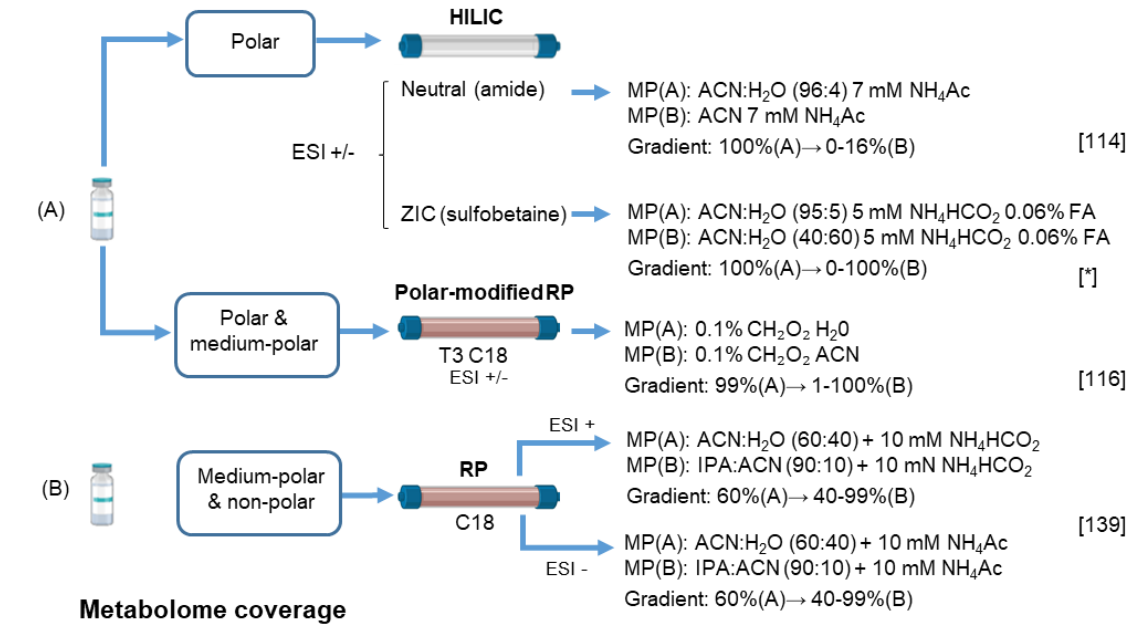
Figure S9. Recommended protocol and technical aspects for tissues





Metabolome coverage

ESI	HILIC (Log P < 1)	RP modified (-3 < Log P < 7)	RP (Log P > -1)
+	**181** metabolites: aas, carbohydrates, acylcarnitines, amines...	**325** metabolites: steroids, acylcarnitines, phospholipids, heterocyclic comp.,aas, amines...	**256** metabolites: steroids, heterocyclic comp., benzenoids, aas...
-	**47** metabolites: carbohydrates, organic acids, Faz, BAs...	**122** metabolites: BAs, phospholipids, ecosanoids, FAs, organic acids, carbohydrates...	**109** metabolites: BAs, Fas, phospholipids, ecosanoids, organic acids...
+/-	**254** metabolites: aas, carboxylic acids, heterocyclic comp., nucleotides, carbohydrates...	**356** metabolites: aas, carboxylic acids, FAs, BAs, steroids, heterocyclic comp., nucleotides, benzenoids, carbohydrates...	**252** metabolites: FAs, BAs, steroids, heterocyclic comp., organic acids, benzenoids, flavonoids...

aas: amino acids; FAs: fatty acids, BAs: bile acids

Tips

- Remove residual blood as quickly as possible and freeze the sample instantly to ensure homogeneous freezing
- Weigh on dry ice to avoid thawing tissue
- For dry grinding, the sample is weighted after grinding. For wet-extraction, the sample is weighted before adding the solvent
- For dry grinding temperature must to be controlled to prevent thawing. For wet extraction keep temperature below 4ºC
- Use ice-cold extraction solvent to prevent enzymatic metabolite degradation
- Centrifuge at least 2x at max speed at 4 °C to ensure that no debris is transferred to the HPLC vial
- The homogenization conditions must be adapted to the tissue (beads / speed / cycles)

[*] Recommended conditions based on our experience