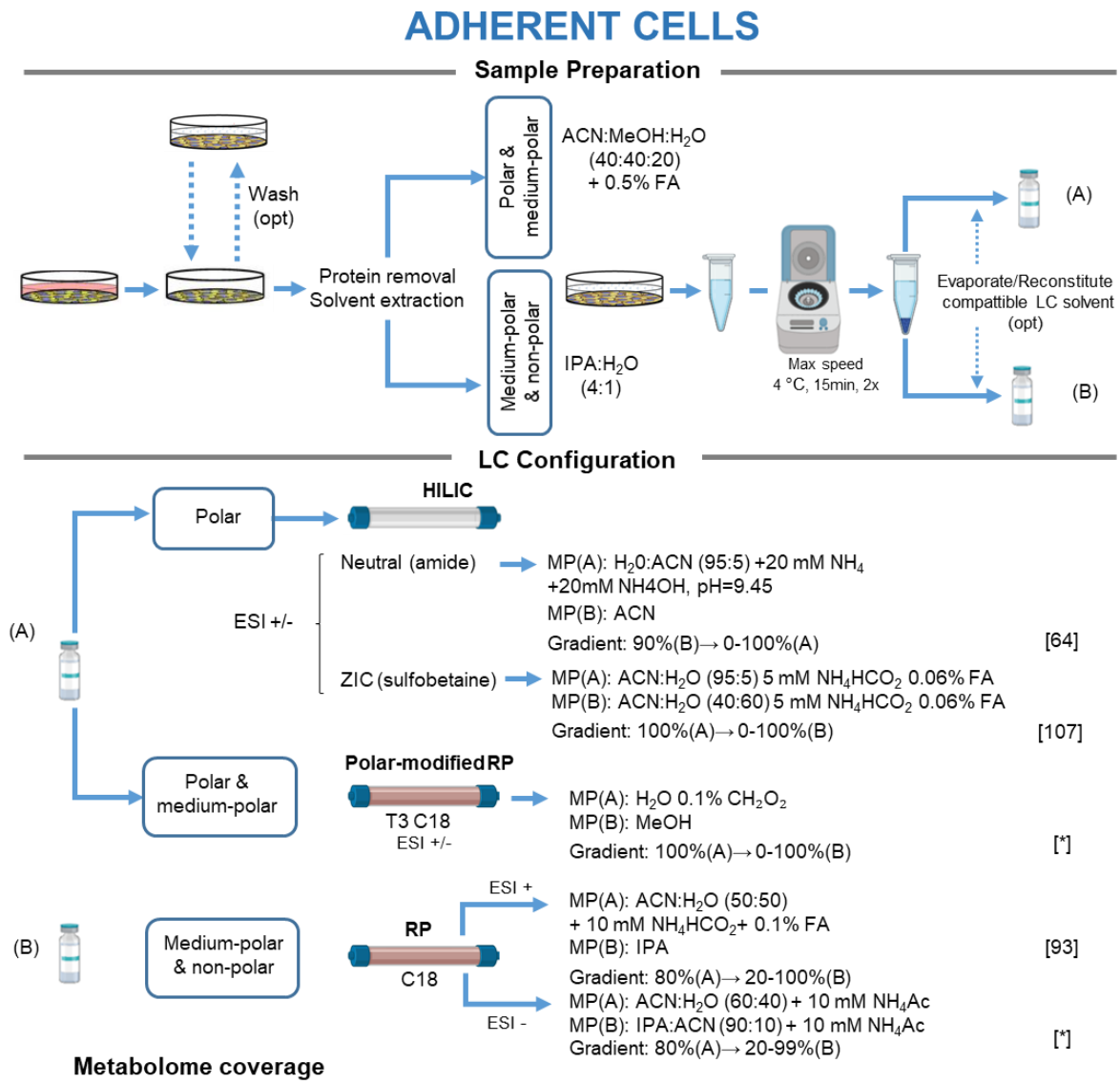
Figure S8. Recommended protocol and technical aspects for adherent cells



Metabolome coverage

ESI	HILIC (Log P < 1)	RP modified (-3 < Log P < 7)	RP (Log P > -1)
+	**379** metabolites: heterocyclic comp., aas, carbohydrates, benzenoids, acylcarnitines...	**1089** metabolites: heterocyclic comp., benzenoids, acylcarnitines, steroids, phospholipids, aas, amines...	**1001** metabolites: heterocyclic comp., benzenoids, steroids, aas, organic oxygen...
-	**110** metabolites: carbohydrates, FAs, organic acids...	**268** metabolites: FAs, ecosanoids, phospholipids, carbohydrates, benzenoids, organic acids, flavonoids...	**241** metabolites: FAs, ecosanids, phospholipids, benzenoids, flavonoids, organic acids...
+/-	**486** metabolites: aas, heterocyclic comp., nucleotides, carbohydrates, benzenoids...	**973** metabolites: flavonoids, heterocyclic comp., FAs, sterpoids...	**818** metabolites: FAs, steroids, BAs, flavonoids, benzenoids, aaa...

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

Tips

- Ideally adjust extraction volume to cell number or packed cell volume, plan ahead to have 1 replicate per condition
- Perform the optional wash step only if contamination from media metabolites is relevant.
- Once the media is removed work as quickly as possible to prevent alterations in the metabolome, ideally process a max of 6 samples at a time until the step where the extraction solvent is added.
- 500 µL of extraction solvent are enough to cover the surface of a 6-well plate and usually further sample concentration is not usually required.
- Use ice-cold extraction solvent, and incubate 5 min on ice to favor cell lysis while preventing enzymatic metabolite degradation.
- The cell monolayer can be scraped and transferred to the Eppendorf tube together with the supernatant metabolite extract.
- Centrifuge at least 2x at max speed at 4 °C to ensure that no cell debris is transferred to the HPLC vial.

[*] Recommended conditions based on our experience